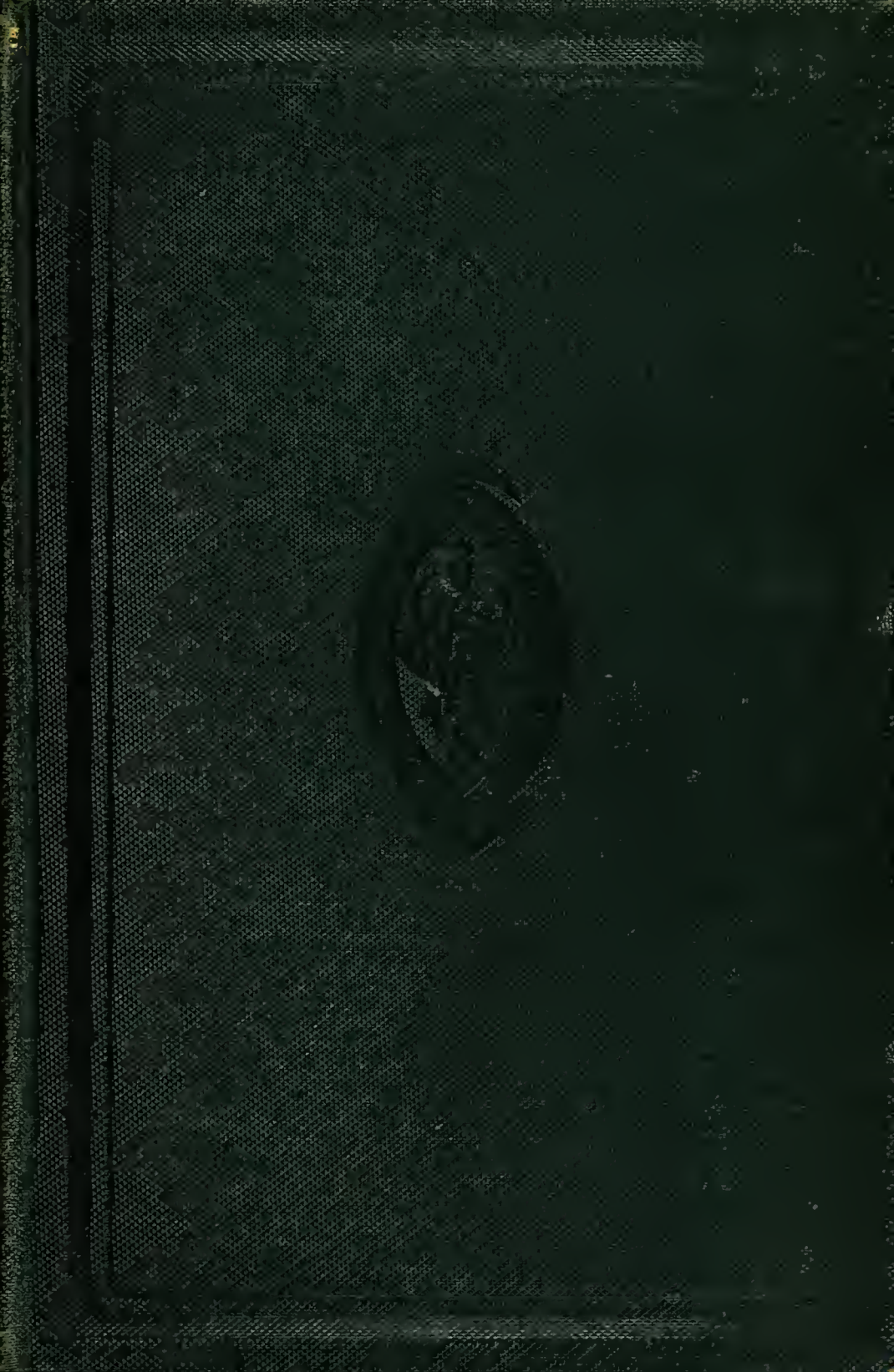

THERAPEUTIC IMMUNIZATION

IN ASYLUM AND GENERAL PRACTICE

BY

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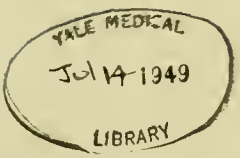
NEW YORK

WILLIAM WOOD AND COMPANY

EDINBURGH

E. & S. LIVINGSTONE

1921



Preface

MEDICAL treatment at the present day is chiefly palliative. The aim of scientific medicine should, however, be to arrest the causes of disease. This is precisely the great purpose of *Therapeutic Immunization* and its distinctive claim to consideration. It is not, however, in medicine alone that we have to deplore the too frequent adoption of the short-sighted policy of being content with the application of palliative measures to existing ills, instead of seeking to arrest them at their source. The same kind of folly is often manifest in national and local government and in all sorts of philanthropic activity. Millions are yearly spent in mopping up, when a comparatively small sum wisely applied to the work of stopping the leak would do infinitely more to mitigate the evil. In only one or two directions is bacteriology being properly applied at the present day. In general medicine and surgery it is not being utilized as it ought to be in the interests of the patients. To point out some of the respects in which medical and surgical practice falls short of what is attainable in the application of modern bacteriology to the investigation, treatment and prevention of common maladies, and to urge a wider recognition of the value of therapeutic immunization, are the chief aims of this book.

The arrangement of the subject matter has been carefully considered, and an endeavour has been made to give a systematic account of all that concerns therapeutic immunization. There is obviously overlapping, but it is worth while, I think, even at the cost of occasional repetition, to view a subject like this from several different points. The alternative to such a method is to burden a smaller number of chapters with details of diverse nature, with consequent loss of clearness.

It was, I felt, more important to endeavour simply to collect all that seemed most valuable in the work of others on the subject of therapeutic immunization, than to take note of what appeared to me to be mistakes and shortcomings. Both the science and practice of therapeutic immunization are still at an early stage in their development, notwithstanding the confident and satisfied tone that has too frequently been adopted by writers on the subject.

In this book there are incorporated the results of many years of research. Numerous facts new to science are

recorded, and many heterodox opinions are expressed that are not likely to be accepted without a fight.

I have many acknowledgments to make. First of all, my thanks are due to the Board of the Laboratory of the Scottish Asylums (and especially to its present Secretary and Treasurer, Dr Robert B. Campbell), in whose laboratory the researches upon which this book is based have been carried out. I would take this opportunity of recognizing the confidence the members of the Board have shown by allowing me to pursue without question those lines of research that seemed to me most profitable. Many years were occupied by what was little more than the laying of foundations, but now the superstructure is, I believe, beginning to rise, and the achievement of the aim of my work, the attainment of methods of curing and preventing some of the common forms of mental disorder, is within sight, and has indeed already, in some small measure, been realized. I have carried this matter considerably further than would appear from what is written in the book, and I would refer the reader to a paper on Dementia Præcox, already communicated to the Medico-Psychological Association, which will appear in the October number of *The Journal of Mental Science*.

The mere enumeration of the names of those who have given me valuable help in my labours during the past ten years would occupy several pages. To most of the superintendents of the institutions associated in the maintenance of the Laboratory of the Scottish Asylums, I am indebted in this way, as well as to many of their past and present assistants. Among these I would specially mention Dr Charles A. Crichlow, assistant to Dr Patrick Steele, at Roxburgh District Asylum, and Dr E. M. Johnstone, Dr Bell Emslie, Dr William McAlister and Dr Neil McLeod, assistants to Professor George M. Robertson. For opportunities of investigating many instructive cases of nervous disorder outside of asylums, I have to thank especially Dr Arthur Wilson, Dr A. Fraser Lee, Dr Robert Robertson, Dr George Henderson (Coldstream), Dr Logan (Ecclefechan) and Dr John McLaren (Edinburgh). I have also to acknowledge important practical help received from Mr J. J. Ritchie in the compilation of the section on bacteriological methods. I have to thank Miss M. Pairman and Dr Arthur Wilson for the time and trouble they have devoted to the correction of the proofs. Lastly, I owe a special word of thanks to Mr Thomas Stephenson, Editor of *The Prescriber*, for very useful practical advice in the compilation of the index.

Contents

Therapeutic Immunization

CHAPTER I

INTRODUCTION

THERAPEUTIC IMMUNIZATION consists in the specific stimulation of the defensive mechanisms of the body against actual bacterial attack with the object of destroying, or expelling, the invaders, and restoring the patient to health. Its profitable application is already extremely wide, and is certain to extend.

Protective, or **prophylactic inoculation** consists in the specific stimulation of the same defensive mechanisms against a particular bacterium with the object of strengthening them for a more or less prolonged period, and so preventing a future attack by this bacterium. Its applications are already numerous and very successful, but its use against many common infections, such as those causing influenza, whooping-cough, and other acute catarrhs of the respiratory tract, is still far too limited. At the end of a course of therapeutic immunization, when the attacking bacteria have been eradicated, we may carry out the same process of protective inoculation, with the object of further strengthening the defences, and so preventing reinvasion.

Serum therapy consists in the introduction into the body of serum containing specific antibodies capable of neutralizing the toxins produced by the corresponding infecting bacteria. Its profitable applications are at present very limited. The chief diseases in which it has been successfully used are diphtheria, cerebro-spinal fever, tetanus and other wound infections by sporing anaerobes.

In the course of the study of infective diseases of known bacterial origin by thousands of workers, the existence of a

complex mechanism of defence against the invaders has been made apparent to all, and it was therefore natural that the thoughts of many should be turned to the possibility of artificially stimulating this mechanism for therapeutic purposes. The defensive processes were seen to be of the nature of a special response to the action of a particular bacterial toxin, and it was a very simple inference that they should be capable of stimulation by the introduction of measured doses of the same toxin into the body. Too much credit has often been given to the efforts of early workers in this department of therapeutics. These efforts were commendable, but only to a limited extent. They consisted chiefly in the common-sense application of discoveries already made by others. They were necessarily at first tentative; they were often misdirected. There have been many pioneers in the field of therapeutic immunization, but as was inevitable in a matter so complex and difficult, their work has frequently been marred by unfortunate mistakes, by failure to perceive what ought to have been obvious, and by grave shortcomings. The credit due to some of these pioneers is certainly great, but it will be possible to appraise it correctly only in historical retrospect, when the science and practice of therapeutic immunization have advanced much beyond the position they have as yet reached.

At the present day, therapeutic immunization does not hold a place in medical practice in the least commensurate with the frequency and importance of bacterial infections as causes of illness, or in keeping with its value as a method of treatment. The reasons for this state of affairs are pretty clear.

1. Systematic instruction in therapeutic immunization is not given in the medical schools. Consequently, a knowledge of this method of treatment must be acquired from occasional papers in the journals and from a few books, which, however valuable they may be, do not take the place of systematic teaching and practical demonstration. Most doctors do not take the trouble, or have not time, to gain even the little knowledge of the subject that may thus be obtained. Hence it results that few practitioners are able to understand the

significance of the facts detailed in a modern bacteriological report in any but the simplest cases, or to carry out immunization correctly when supplied with properly prepared and accurately standarized vaccines. The few to whom this statement does not apply have been for the most part their own instructors; they are specially keen and intelligent observers, men of enterprise and courage, who have grasped and applied a great therapeutic principle, and who have not allowed their faith in it to be destroyed by occasional failure.

2. There has been an improper and random use of vaccines by general practitioners and others, with, as was inevitable, disappointment in the results obtained. Stock vaccines, vaunted by those who have a commercial interest in their extensive employment, have been very largely administered without knowledge of the actual bacterial infections from which the patient was suffering. Anyone who has made extensive bacteriological investigations in cases of the kind that have been treated in this way knows very well that the composition of these vaccines can be regarded as appropriate for the therapeutic immunization of only a very small proportion of the patients. The chief fault is one of defect; infections of the alimentary and respiratory tracts are almost always complex, and one or several important elements in the pathogenic flora are generally not represented in the stock vaccine. Added to this, there are the facts that named pathogenic bacteria have numerous independent strains, and that a vaccine made from one strain will not protect against another. Stock vaccines have their appropriate use, as is maintained in this book, but the fact remains that they have been grossly misused in the past, and are still being misused to the great discredit of the science and practice of therapeutic immunization. Wrong methods of standardization, and lack of knowledge regarding focal reactions, hypersensitiveness and the principles of correct dosage have all contributed to the number of unsatisfactory results. Occasional accidents, for which the person who prepared the vaccine, or the doctor himself, and not therapeutic immunization, was to blame have led to the loud condemnation of vaccines in general. Many instances of the kind have come under my notice. Had drugs been used in the same improper

and random fashion, they must similarly have fallen into disrepute as therapeutic agents. In view of some of the facts that have come to my knowledge regarding the improper and unskilled use of vaccines, I am surprised, not that therapeutic immunization is unpopular with doctors, but rather that the legislature has not intervened to prohibit its employment altogether.

3. Therapeutic immunization ought to be based upon a complete and accurate bacteriological analysis of the pathogenic flora of the infected area in the case; if it is not, we are simply shooting a bow at a venture. How often is such a bacteriological examination carried out as a preliminary to this form of treatment? As yet, only very rarely. Even when a bacteriological examination is made, the analysis is very commonly defective, for the fact must be stated, however distasteful it may be to some, that the orthodox bacteriological methods of the text-books are inadequate for the making of the kind of bacteriological analysis that is required for therapeutic immunization. For example, it is certain that incomplete analysis of the pathogenic flora must often have been made in the past by those who have not systematically used blood-media and anaerobic methods. Incomplete bacteriological analysis must inevitably bring even autogenous vaccines into discredit. Very many instances of this kind, some of them truly deplorable in their consequences, have come to my knowledge.

4. The methods of standardizing vaccines in millions, and by the degree of opacity, which are to this day the orthodox ones, are grossly inaccurate, making administration of correct doses extremely difficult and even impossible. The general adoption of one or other of these inexact methods has had unfortunate consequences that have contributed to the present discredit of therapeutic immunization. I have known of several cases in which a vaccine was so inaccurately standardized that the first dose administered, supposed to be small and safe, made the patient extremely ill. An experience of this kind naturally leads the doctor to conclude that vaccines are dangerous things that had best be avoided.

5. Therapeutic immunization has been placed at a disadvantage by the inadequate teaching regarding the im-

portance of bacterial infection as a cause of common diseases. The bacterial origin of such maladies as tuberculosis, typhoid fever, diphtheria, tetanus, plague and cholera is universally recognized, but the facts regarding the part played by bacteria in the causation of the maladies that doctors have to deal with every day, such as acute and chronic nasal and post-nasal catarrhs, bronchitis, asthma, pyorrhœa alveolaris, chronic pharyngitis, rheumatism, rheumatoid arthritis, anæmia, chronic intestinal catarrhs, and neurasthenia (to name only a few), are understood only in a vague way. It is even still the fashion with many to deride bacteria as the result and not the cause of the malady with which they are associated. When such lack of knowledge prevails, it is impossible that bacteriological investigation and therapeutic immunization can be regarded as important aids in the treatment of disease. An example may make this clearer. Medical practice is still at a stage in which, when a doctor sees a case of illness associated with pyorrhœa alveolaris, he recognizes the importance of the pyorrhœa and probably orders all the teeth to be removed, but he has no accurate or useful knowledge of the infective processes concerned, and no thought of the possibility of curing the morbid condition by ascertaining the exact nature of the infections in the case and suppressing them by appropriate therapeutic immunization. Yet, by adopting these measures, he might, with the co-operation of a dentist, save the patient's teeth and their owner the pain and risks of an operation of considerable gravity. A similar state of affairs prevails with regard to a long list of other common maladies. Resort is rarely had to bacteriological investigation, and patients thus often lose the benefit that might be received from properly applied therapeutic immunization.

It is one of the chief purposes of this book to show that the present unpopularity of therapeutic immunization is undeserved. I have confidence that the day will soon come when it is regarded with general respect, and applied rationally and correctly in the treatment of appropriate cases. Then will come the need for special laboratories in which the kind of bacteriological analysis that case investigations require can be carried out without undue expense being

incurred by the patients. How can such laboratories be equipped and staffed? Public and private enterprise will be capable of providing the equipment; the real difficulty is the staffing. The bacteriological analysis of an average case requires from six to twelve hours' work by a skilled analyst, and two or three hours more must be expended upon making a report on the case and preparing and dispatching the vaccines. Now, if all this work has to be done by a doctor, whose remuneration must be regulated by the amount of capital expended upon his professional education, the cost of therapeutic immunization must be such as to be prohibitive to the majority of patients who ought to benefit by this method of treatment. I wish therefore to point out that it is not necessary that the bacteriological analyst should be a person possessing a medical degree. This has already been proved in the Laboratory of the Scottish Asylums. Anyone, of either sex, with an aptitude for scientific work, who will devote a year to training in a laboratory in which bacteriological examinations for the purpose of therapeutic immunization are constantly being made, can acquire the necessary knowledge and skill, and carry out every detail of the analysis, as well as prepare and standardize the vaccines. The investigations of each assistant will be supervised by a bacteriologist who possesses a medical degree, who will write the report, and decide (it may be in consultation with the doctor in charge of the case) what vaccines are to be prepared. Under some such arrangement, therapeutic immunization will be brought within the reach of all who can benefit by it.

I append here a list of some of the books and special papers to which reference is made in the succeeding chapters.

Literature References

(1) R. W. Allen. *Vaccine Therapy: Its Theory and Practice.* Fourth edition, 1912.

(2) R. W. Allen. (*a*) "The Respiratory Tract in Health and Disease." *Journal of Vaccine Therapy*, 1912.
(*b*) *The Bacterial Diseases of Respiration, and Vaccines in their Treatment.* 1913.

(3) R. W. Allen. *Practical Vaccine Treatment for the General Practitioner.* 1919.

(4) Muir and Ritchie. *Manual of Bacteriology.* Seventh edition, 1919.

(5) R. Tanner Hewlett. *A Manual of Bacteriology, Clinical and Applied.* Fourth edition, 1911.

(6) R. Tanner Hewlett. *Serum and Vaccine Therapy.* Second edition, 1910.

(7) W. D'Este Emery. *Immunity and Specific Therapy.* 1909.

(8) William Cecil Bosanquet and John W. H. Eyre. *Serums, Vaccines and Toxins in Treatment and Diagnosis.* Second edition, 1909.

(9) M. H. Gordon. Abel's *Laboratory Handbook of Bacteriology.* Second English edition, 1912.

(10) Francesco Testi. *Microbiologia pura ed applicata e tecnica microbiologica.* 1911.

(11) W. M. Crofton. *Therapeutic Immunisation. Theory and Practice.* 1918.

(12) Jungano et Distaso. *Les Anaerobes.* 1910.

(13) P. N. Panton. *Clinical Pathology.* 1913.

(14) Clive Riviere and Egbert Morland. *Tuberculin Treatment.* Oxford Medical Publications, 1912.

(15) Sir A. E. Wright. "The Principles of Vaccine Therapy." *The Lancet,* 17th and 24th August 1907.

(16) Sir A. E. Wright and Others. "Studies in Connection with Therapeutic Immunisation." *The Lancet,* 2nd November 1907.

(17) Lewis C. Bruce. *Studies in Clinical Psychiatry.* 1906.

(18) W. Hale White and John W. H. Eyre. "Results of a Year's Use of Vaccines." *The Lancet,* 5th June 1909.

(19) F. W. Andrewes and T. J. Horder. "A Study of the Streptococci Pathogenic for Man." *The Lancet,* 15th, 22nd and 29th September 1906.

(20) Benham. "Common Colds." *Brit. Med. Journ.,* 28th August 1909.

(21) Nathan Raw. *Brit. Med. Journ.,* 22nd December 1901.

(22) R. J. M. Buchanan. "The Pathogenesis and Treatment of Rheumatic Fever and Chorea." *The Journal of Vaccine Therapy,* January 1912.

(23) Sir David Semple. "The Vaccine Treatment of Typhoid Fever." *The Journal of Vaccine Therapy,* February 1912.

(24) S. P. Shivdas. "Observations on Treatment by Bacterial Vaccines." *The Journal of Vaccine Therapy,* April, May, 1912.

(25) Henry A. Craig. "Observations on 395 cases treated with Autogenous Bacterial Vaccines." *The Journal of Vaccine Therapy*, November 1912.

(26) Robert Robertson. "Vaccines in General Practice." *The Prescriber*, 1920.

(27) Sir William B. Leishman. "The Results of Protective Inoculation against Influenza in the Army at Home, 1918-1919." *Brit. Med. Journ.*, 14th February 1920.

(28) W. H. Wynn. "The Use of Tuberculin in the Treatment of Pulmonary Tuberculosis." *The Journal of Vaccine Therapy*, December 1912.

(29) W. H. Wynn. "Protective Inoculation against Influenza." *Brit. Med. Journ.*, 21st February 1920.

(30) W. H. Wynn. "The Early Administration of Vaccine in Pneumonia." *Brit. Med. Journ.*, 13th March 1915.

(31) W. H. Wynn. "The Vaccine Treatment of Septicæmia." *The Journal of Vaccine Therapy*, September 1912.

(32) W. D'Este Emery. *The Journal of Vaccine Therapy*, March 1913, page 84. (Micrococcus Rheumaticus.)

(33) Butler Harris. "The Therapeutic Value of the Pneumococcus Vaccine in the Treatment of Pneumonia." *Brit. Med. Journ.*, 26th June 1909.

(34) Avery, Chickering, Cole and Dochez. "Acute Lobar Pneumonia." Rockefeller Institute for Medical Research, Monograph No. 7.

(35) Richard R. Armstrong. "The Serological Characters of Disease-producing Pneumococci." *Brit. Med. Journ.*, 19th February 1921.

(36) Cecil and Austin. *Journal of Experimental Medicine*, 1st July 1918.

(37) R. W. Allen. "Some Thoughts on Tuberculin and Tuberculin Therapy." *The Journal of Vaccine Therapy*, August 1913.

(38) W. D'Este Emery. "A Note on the Dosage and Dilution of Tuberculin." *The Journal of Vaccine Therapy*, February 1913.

(39) Kraepelin. *Dementia Præcox and Paraphrenia*. Trans. by R. Mary Barclay. 1919.

(40) Kraepelin. *Manic-Depressive Insanity and Paranoia*. Trans. by R. Mary Barclay. 1921.

(41) Bianchi. *Trattato di Psichiatria*, 1901.

(42) Eugenio Tanzi e Ernesto Lugaro. *Trattato delle Malattie Mentali*, vol. i., 1914; vol. ii., 1916.

(43) Bayard Holmes. "Recoverability from Dementia Præcox." *American Medicine*, January 1918.

(44) W. Ford Robertson. "The Pathology of Insanity." Green's *Encyclopedia and Dictionary of Medicine and Surgery*, 1918.

(45) W. Ford Robertson. "Influenza: Its Cause and Prevention." *Brit. Med. Journ.*, 21st December 1918.

(46) W. Ford Robertson. "The Infective Factors in Some Types of Neurasthenia." *Journal of Mental Science*, January 1919.

(47) W. Ford Robertson. "A Starch-splitting Bacterium found in Cases of Diabetes Mellitus." *The Journal of Pathology and Bacteriology*, October 1919.

(48) W. Ford Robertson. "The Relation of Infections to Mental Disorders." *Journal of Mental Science*, July 1920.

CHAPTER II

BACTERIAL INFECTION AS A CAUSE OF DISEASE

THE growth of bacteriology during the past forty or fifty years has been one of the greatest marvels of modern science Nevertheless, in some respects it has been strangely one-sided. The infective origin of many highly important diseases has been fully demonstrated, and the pathogenic action of bacteria and the mechanism of defence against them have been made the subjects of exhaustive and very successful investigations, but the study of the part played by bacterial infection in the causation of what may be classed, for convenience, as common diseases has been comparatively neglected. All medical men have precise knowledge of the specific infective origin of such diseases as tuberculosis, diphtheria, typhoid fever, tetanus, plague and cholera, but the same cannot be said of them regarding, for example, chronic bronchitis, rheumatism, rheumatoid arthritis, pyorrhœa alveolaris, chronic catarrh of the Eustachian tubes and eczema, the bacterial origin of which is no less certain. The importance of bacteria in the causation of such maladies is even denied. When it is contended that they are of infective origin an answer is given that amounts to this: There is no proof that the bacteria you cultivate from the discharges in these cases are the cause of the patient's illness; there is no evidence from animal experiment, such as that upon which rests our knowledge of the causation of tuberculosis, diphtheria and tetanus. The exclusive faith still displayed in the evidence of experiments made upon laboratory animals is greatly to be deplored. It has done much to retard the progress of medicine. There is available evidence of the pathogenic action of particular species of bacteria in common diseases far more definite and conclusive than that which animal experiment could possibly furnish. It consists in the observation of focal reactions and of the

effects of therapeutic immunization. Every case dealt with by accurate bacteriological investigation and therapeutic immunization has its instructive lesson, establishing some point of etiology. When personal experience of the application of what may be called the method of focal reaction and therapeutic immunization has run into thousands of cases, the accumulated evidence must take shape in definite and solid conclusions. The final test of the validity of these conclusions is their applicability to fresh cases of a similar kind, and this test they have been found to stand. The experiences of many workers in this field of investigation can now be compared and conclusions of the utmost importance for practical medicine can be drawn from them.

The available evidence regarding the pathogenic action of various species of common bacteria, and the part played by simple or multiple infections in the causation of very many maladies of the kind that the practitioner sees almost every day, will be adduced in its proper place. Here it may be stated that, within the last three or four years, the number of common maladies that have been shown, by the methods to which I have alluded, to be due essentially to bacterial infection has been largely augmented. It may surprise some to know that it now includes most types of neurasthenia, exophthalmic goitre, rheumatoid arthritis, pernicious anæmia, duodenal ulcer and diabetes mellitus. Further, it has now been shown by very numerous experiences that many cases of chronic illness, with symptoms that do not permit of very exact classification of the malady, are dependent upon bacterial infection. A systematic investigation reveals gross abnormalities in the bacterial flora, the administration of autogenous vaccines provokes focal reactions, and under continued therapeutic immunization the patient is restored to health. Numerous examples of this nature are recorded in subsequent chapters. In my own hands, bacteriological and therapeutic investigation of this kind has also been carried into the field in which it was primarily intended to seek its testimony—namely, that of mental diseases. Such extension could not have been safely made until the common field had been well surveyed. It is now possible to define

with some exactitude the part played by bacterial infection in the complex etiology of insanity, and already therapeutic immunization has had its triumphs in the treatment and prevention of this form of disorder. This subject will presently be given separate consideration.

Whilst the importance of the part played by bacteria in the causation of disease is at the present day imperfectly recognized, it need not be exaggerated. There are other causes of disease that we cannot afford to ignore, and the complex factors of predisposition, natural and acquired resistance, and temporary weakening of the defences have also to be considered. Indeed, unless these associated factors in the causation of disease are taken into account, the true meaning of bacterial infection cannot be understood. A brief consideration of them is therefore not out of place here.

The determining causes of disease may be grouped under the headings of bacteria, protozoa, animal parasites, toxic substances and traumatisms, in the widest sense, and including psychical traumatisms, the mode of action of which is similar to that of physical traumatism.

It is necessary to limit the definition of disease. We continually stand on our defence against a host of inimical forces in our environment. We have a first line of defence, consisting of the skin and mucous membranes, to an important but limited extent impermeable by bacteria and toxins, and a second line of defence consisting in the complex forces of natural and acquired immunity. Disease, in a sense much narrower than that in which the term is commonly used, is the reaction of the living body to an inimical force that has penetrated the first line of defence. It is the second line of defence in action, sometimes fighting well and successfully, at others overpowered and retreating in confusion. We are made aware of the struggle by feelings of malaise, by pain, rise of temperature, local inflammation and its consequences, disordered heart action, and innumerable other signs that vary in accordance with the character of the inimical force, its point of attack, and the special reactive qualities of the body invaded. The fluctuating battle may wage for months or years, as, for example, in pulmonary tuberculosis.

The inimical force may be entirely overcome and normal conditions restored, but very commonly permanent injury is done to various organs, impairing their future functional activity. Often it is simply the disorder of metabolic processes thereby entailed that we see, long after the inimical forces that caused it have spent themselves. A body in this position cannot be regarded as normal, but is properly termed diseased. Yet we must distinguish clearly between disease, which is an active process, and the structural changes produced by disease. These structural changes may be so slight that complete compensation is established, or they may be incompatible with the future normal performance of the physiological processes of the body. An example of each kind may be seen in some persons who have suffered from exophthalmic goitre. There may be complete suppression of the toxic process that caused the hypertrophy of the thyroid gland and restoration of the organ to its normal condition ; in other cases, the disorder of the thyroid gland may have been so serious as to be incompatible with its normal action after cessation of the intestinal toxic action, and a condition of hypothyroidism remains, entailing permanent disorder of the bodily chemistry, fortunately now amenable to treatment by thyroid extract.

A qualifying factor that requires always to be taken into consideration is that of individual predisposition. It would have been remarkable, indeed, if every human being and every animal had been exactly alike in its power of resistance to inimical forces in its environment. It is inevitable, in the nature of things, that there should be considerable range of variation in this respect. The range is very wide when different animal species are compared ; it is much narrower, but still wide, in the human race. The simplest example is, perhaps, the defective resistance of some families to the attack of the tubercle bacillus, compared with the perfect power of resistance exhibited by others. The matter is, however, far from being a simple one. The special manifestations of predisposition are numerous, and its factors, which are included in those of natural immunity, are complex and often obscure. Among the phenomena that it has to be called in to account for, in addition to the

hereditary tendency to tuberculosis, are the special incidence in families of such morbid conditions as rheumatoid arthritis, post-nasal infections, eczema, psoriasis, acute and recurrent forms of insanity and dementia præcox. Some forms of predisposition, as, for example, that in rheumatoid arthritis, seem to consist essentially in a special readiness to fix toxins in a particular tissue or organ. Other examples are the somewhat common cases in which the toxins of the influenza bacillus specially affect the heart muscle, or its intrinsic nervous apparatus, and the important group of cases in which the poisons developed by neurotoxic organisms of the diphtheric group fix themselves in particular nervous tissues.

A great amount of misconception seems to exist among medical men regarding the exact position of bacteria in chronic infective conditions of mucous membranes. The general conception seems to be that the bacteria are lying only at the surface and in the secretions. Now, this is a very erroneous and unfortunate belief. If the bacteria were lying merely in the surface secretions, they would be harmless. They are really in the tissues, for the most part near the surface. It is easy to convince anyone of this by showing a properly stained microscopical preparation of a scraping from a pharyngeal wall, the seat of chronic infection, or of a spongy gum associated with pyorrhœa alveolaris. Gram-fast bacteria, most commonly streptococci, can be seen lying between the epithelial cells, far below the surface, as well as among the subjacent connective tissues. Such bacterial invasion of the tissues can often be demonstrated also in other situations, and more especially the genito-urinary tract, scrapings from which often show most striking pictures of the lodgment of diphtheroid and coliform bacilli among the epithelial cells.

The Relation of Infections to Mental Diseases

The study of the pathology of mental diseases is beset by very special difficulties. The organs concerned—namely, the association centres of the brain—are far more highly elaborated than any others, and the manifestations of their

functional activity are of quite a different order from those of any other living structure. It is especially the latter circumstance that has obscured the fundamental facts and prevented the adoption of a view of the pathogenesis of mental diseases that makes it possible to apply to them the principles of general pathology.

The matter has hitherto been regarded almost exclusively from the point of view of psychology. To the orthodox psychologist it is absurd to regard mind as merely an expression of the functional activity of the brain, corresponding to the functional activity of the stomach or kidneys. It has been laid down by him that "The brain is not the organ of mind in the sense in which it is the organ of sensori-motor activity," and that "There is no evidence to support the position that the mind is a function of the nerve fibres and nerve cells." Modern science, which founds its conclusions purely upon evidence, is bound to take a diametrically opposite view. That mind is an expression of the functional activity of the association centres of the brain is supported by a mass of anatomical, physiological and pathological evidence of a conclusive nature. The matter is important, for it is only by regarding mind in this way that it becomes possible to bring the pathology of mental diseases into line with general pathology, and until this is done, our knowledge of mental diseases must remain disinherited of its share in the magnificent patrimony of the modern science of disease, which includes many therapeutic benefits.

I have elsewhere (44) endeavoured to show that the principles of general pathology can be applied to the pathology of insanity. Although we are concerned in this book only with the part played by infections in the causation of mental disorders, it is necessary, in order that the nature and importance of this part may be understood, to have a clear idea of all the factors in their pathogenesis. A brief review of the subject is therefore required here. It is necessary to begin with fundamental facts.

Every living thing is the product of two distinct factors: heredity and environment. Heredity is only a moulding force, varying slightly in its potentialities in different stocks in the same species. In co-operation with environmental

forces it builds up from the germ cells a highly complex organism. The individual thus developed is a vital reactive mechanism; every vital phenomenon it is capable of exhibiting is of the nature of a response to external stimuli. This is true of the being as a whole, of the separate organs and of each cell composing the tissues. For example, the functional activity of a glandular organ, such as the stomach, is purely a response to stimuli from its environment, which includes the other organs and tissues of the body. The same principle applies to the nervous organs, and not only to the lowest, but to the highest. The brain is a reactive mechanism of extreme complexity, commonly elaborated to an extraordinary degree by education. Consciousness we can understand only as a concomitant of reaction in the associative or psychical centres. When we hear the ring of the telephone bell and go to the instrument to answer the call, we perform a series of complex reflex actions induced, firstly, by the sound of the bell, and secondly, by the representations awakened thereby in the psychical centres, and with every step of the series of reactions there flows a stream of consciousness. Normal mental reactions are strictly conditioned by the integrity of the central nervous mechanism. If this mechanism is damaged, its functional reactions must be abnormal. The mechanism becomes damaged either by traumatism or as a consequence of disease. The potency of traumatism in perverting or arresting mental action hardly needs illustration. The part capable of being played by disease requires consideration.

As already maintained, disease is a reaction on the part of the living body to an inimical force that has penetrated its first line of defence, constituted by the skin and mucous membranes. Pathogenesis is an account of the defensive struggle, of the forces engaged on either side, and of the havoc often wrought in the course of the battle. As results of this struggle and of the exposure of delicate structures to toxic actions, irreparable injury may be sustained by various organs and tissues, in consequence of which their functions are afterwards imperfectly performed and conditions of auto-intoxication are induced. Some tissues have very little power of active self-defence, and their escape from injury

depends upon their relative invulnerability. If a toxin is circulating in the blood, it will fix itself in any substance for which it has a chemical affinity. Now, there are many toxins, and some of them of bacterial origin, that have special affinities for nerve cells. The liability to be injured by such neurotoxins is, however, not the same in all nerve cells. There are wide local and individual differences, dependent upon congenital and acquired qualities. Special vulnerability to toxins on the part of the nerve cells of the association centres in some stocks appears clearly to be at least one important factor included in hereditary predisposition to insanity. Two distinct effects upon the association centres must be recognized. During the height of the toxic action the metabolic processes occurring in these centres are seriously disturbed; mental reactions are correspondingly disordered and consequently there is confusion, excitement, depression or stupor. If the toxic action subsides, there may be complete recovery on the part of the tissues, in which case the mental reactions again become normal. In other cases, there is irreparable destruction of many neurons, and the centres are permanently damaged; the mental reactions therefore remain more or less abnormal. Thus regarded, mental disorders are abnormal reactions of an associative mechanism that is damaged by active, or former disease, or by traumatism, or that is defective owing to some developmental fault. We can now understand the fundamental difference in the manner in which toxins cause disorder in the intellectual functions, respectively in acute confusional insanity and in dementia præcox during its later stages. In the first, the toxic storm still rages, perverting the metabolism of the nervous centres and therefore also their delicate reactive qualities; in the second, the toxic storm has passed over, perhaps many years before, leaving a permanently damaged nervous mechanism which can react only abnormally. The brain of an insane person may be likened to a piano, the intricate mechanism of which is broken, rusted or clogged; the player represents the environment to which it can react. Though his skill is perfect, by fingering the keys he can produce nothing but discord.

If we can regard morbid mental phenomena in this way,

we must recognize the enormous practical importance of the many inimical forces that are capable of breaking through the first line of defence of the body and damaging, temporarily or permanently, the delicate reactive mechanism of the association centres. We are here concerned with only one group of such inimical forces—namely, pathogenic bacteria and their toxins. Some of the evidence that has accumulated is detailed in a subsequent chapter. It is derived from the investigation of a series of cases; from the study of focal reactions, which establish the fact of the pathogenic action of the bacterium and often reveal much regarding its special toxic properties; and, lastly, from the observation of the effects of therapeutic immunization.

CHAPTER III

IMMUNITY

THE way in which the body defends itself against the attack of pathogenic bacteria and protozoa is a matter of fundamental importance to scientific medicine, and hundreds of able workers, recognizing this, have in recent years devoted an enormous amount of patient labour to the elucidation of the extremely difficult problems that the subject presents. A mass of important knowledge has now accumulated, but much that it is desirable should be understood is still obscure. Only a brief outline of the facts regarding immunity that bear upon therapeutic immunization is essayed here, and the reader is referred for fuller information to the discussions of the subject contained in such works as Muir and Ritchie's *Manual of Bacteriology*, Hewlett's *Manual of Bacteriology*, and D'Este Emery's *Immunity and Specific Therapy*. We are concerned almost exclusively with immunity to attack by bacteria, as therapeutic immunization is not yet clearly applicable to diseases caused by protozoa.

Man and the lower animals are continually on their defence against bacterial enemies. The highly organised tissues and fluids of the body are suitable food material for many species of bacteria, some of which are more or less commonly in actual contact with the surfaces of the body. These bacteria would immediately make use of this food material were it not for the mechanisms that serve to repel their attacks. Many species, capable of attacking and breaking down the tissue-elements of the host when a favourable opportunity is afforded, flourish in the natural secretions of the body, thus living at a surface as saprophytes. Such saprophytic bacteria, capable of pathogenic action, occur throughout the alimentary tract, in part at least of the respiratory tract and on the skin. Various other species of pathogenic bacteria, and virulent strains of some perhaps

already present, are liable from time to time to reach the external and internal surfaces of the body. If bacteria invade the tissues, and multiply in them, they quickly produce more or less powerful toxins, which threaten the life of the host. These toxins are formed chiefly as the result of the death and disintegration of many of the bacteria, but, in some instances, also as a vital secretion from them. The various pathogenic species differ widely in the characters of the toxic phenomena and tissue-lesions they produce, as well as in regard to the usual points of their attack. To prevent such attacks, which threaten the life of the host, there are certain complex physiological mechanisms, which, had they been equal to all emergencies, would hardly have attracted attention. Nature is not, however, extravagant in her provision for the bodily defence. She is, indeed, as economical as possible. Such careful balance of defence and of common forms of bacterial attack is a matter of adaptation to environment, which is generally slow and for the most part attained for the race only after a great sacrifice of individuals. As a rule, we find that the defensive mechanisms are just sufficient and little more for ordinary conditions. Under extraordinary conditions, they are apt to prove inadequate, so that some species of bacteria are able to invade, and even to establish themselves in the tissues, to the grave injury of the host. Nature to a large extent compensates for her niggardliness in providing for defence against sudden bacterial attack by a system of reserve forces, capable of being mobilized on short notice.

The ordinary defensive mechanisms are those concerned with natural power of resistance, or natural immunity. The extraordinary forces that are capable of being brought into action when the former are overcome, or in order to meet the attack of new bacterial foes, are those concerned in what is generally termed acquired immunity.

Natural Power of Resistance, or Natural Immunity

Some animals are completely immune to the attack of bacteria that are common causes of disease in other species.

Each animal species has indeed its special susceptibilities to attack by particular bacteria and its immunity to attack by others. The factors upon which these special susceptibilities and immunities depend are complex, and cannot be expressed in any simple terms.

The way in which the body is protected against the common forms of bacterial attack has been ascertained in its main particulars. There is a first line of defence, formed by the skin and mucous membranes. These constitute a barrier, not only against invasions by bacteria and protozoa, but also against the passage of toxic fluids and gases, and of moving bodies of various kinds. The disadvantage that mucous membranes have as compared with the skin, on account of their softness, moist conditions, and higher temperature, is compensated for by the possession of mucous glands, the secretion of which is in itself bactericidal, whilst its outflow tends to sweep minute particles away from the surface. Whenever this first line of defence is penetrated by a force inimical to the welfare of the body, a call is made upon the second line of defence. If this inimical force is bacterial, the polymorphonuclear leucocytes and the large mononucleated cells of the blood, and also the endothelial cells of the invaded tissues, proceed to engulf and digest the invaders. Simultaneously, the extra-cellular digestion of many of the bacteria takes place through the action of a proteolytic ferment, termed complement or alexine, probably derived from the leucocytes. The bacteria may be entirely destroyed by these actions, and the tissues then return to their normal state, excepting perhaps for the effects of proliferation of connective tissue cells, which leads to the formation of some new fibrous tissue. The phagocytic action of the cells and the solvent action of complement may, however, for various reasons, prove inadequate to the task of completely destroying the rapidly proliferating bacteria. The consequence to the host may be death, owing to the action of the bacterial toxins and secondary poisons upon vital organs. This result is, however, commonly obviated by the action of the auxiliary defensive forces concerned in acquired immunity. Indeed, the moment the mechanisms of natural immunity have been roused to special local activity,

these auxiliary forces begin to be mobilized. As a rule, however, they do not gather sufficient strength to be really effective until several days have elapsed. For example, in acute pneumonia of pneumococcal origin, from five to seven days are generally required.

Acquired Immunity

An illness due to a natural bacterial attack, when recovered from, may be followed by immunity to similar attack, lasting for months or years. In some instances this immunity is prolonged, as, for example, after typhoid fever. In others, it is comparatively short, as after an attack of influenza, erysipelas, pneumonia, or diphtheria.

The nature of the special forces that have been brought into action in such cases, in order to expel the invader and to prevent reinvasion by it, have been the main subject of the very arduous labours to which reference has been made. These labours have not been misspent, poorly as they have often been rewarded, for the questions it has been sought to determine by them are of fundamental importance for the future welfare of the human race.

The destruction of the invading bacteria with which the ordinary phagocytic and bacteriolytic forces have been unable to cope is effected, or at least attempted, by the elaboration of specific antibodies of various kinds that either neutralize the bacterial toxins or indirectly effect the destruction of the bacteria themselves. An exotoxin, such as that produced by the bacillus of diphtheria, stimulates the production of an antitoxin which neutralizes it, and, when the toxin is thus disposed of, the ordinary phagocytic and bacteriolytic forces have little difficulty in destroying the invaders. Comparatively few bacteria, however, exert their pathogenic action by means of an exotoxin; the great majority of them become toxic only when their protoplasm undergoes disintegration, liberating what is generally called an endotoxin. Such disintegration occurs as a natural process in the growth of bacteria, as may readily be demonstrated, for example, in a two or three day broth culture of a streptococcus.

It is on account of similar disintegration that the invading pathogenic bacteria mainly exercise their toxic action. The toxins so formed reach the lymphatics and the general circulation, and stimulate the production of antibodies, which are of various kinds. These antibodies are strictly specific in their action, affecting only the kind of bacterium that has provoked their formation. They are, however, incapable of exercising their antibacterial action unaided; they all require the assistance of a ferment contained in the normal blood and probably derived from the leucocytes—namely, the substance known as complement. Among the various kinds of antibacterial substances that have been recognized and studied in their actions are the following:—

Bacteriolysins: specific antibodies that effect the solution of the bacteria.

Agglutinins: specific antibodies that so alter the bacteria that they run together to forms clumps. This phenomenon is significant of more subtle changes detrimental to the life of the bacteria.

Opsonins, stimulins, or bacteriotropins: specific antibodies that act upon the bacteria in such a way as to render them more easily taken up by phagocytes. The normal complement has a similar action; it differs from the specific bacteriotropins in being thermolabile (destroyed at 55° to 60° C.).

Precipitins: specific antibodies that precipitate and so render more or less inert the bacterial toxins.

To these are to be added specific antitoxins to the bacterial endotoxins.

There are probably really three main classes of auxiliary antibacterial action—opsonic, bacteriolytic (bacteriolysins and agglutinins) and antitoxic (specific antitoxins). The first and second may occur in excess of the third, and in consequence there may be temporary increase of the toxæmia, and therefore also of the symptoms of the disease, when the illness is really taking a favourable course. There is much evidence to show that, difficult as the fact may be of explanation on the ground of the known factors of specific immunity, the power of resistance to invasion by a particular bacterium is almost exactly proportional to the degree of tolerance to its

toxin. The explanation may be simply that specific opsonic and bacteriolytic powers are generally increased in the same ratio as the antitoxic power, of which alone we have evidence in observing the tolerance to a given dose of bacterial emulsion. This view must, however, be qualified by the consideration that tolerance of bacterial toxin may be in part an actual tolerance of the poison, as distinguished from power to neutralize it.

Such are the main factors in natural and acquired immunity. It may help to make the matter clearer if their action is further considered and compared, as it is manifested in common forms, first, of acute, and, second, of chronic bacterial infections.

The Defensive Processes against Acute Infections

We may take as examples : (1) an attack upon the mucosa of the upper respiratory tract by *micrococcus catarrhalis*, causing acute coryza, or a common cold ; (2) local invasion of the skin and subcutaneous tissues by *staphylococcus pyogenes aureus*, resulting in the development of a boil ; and (3) an infiltration of the tissues of the tonsils by *streptococcus anginosus*, leading to acute quinsy.

The bacteria, having penetrated the first line of defence, proliferate locally with little restriction for several hours. Many get into the lymphatics, in which, or in the lymphatic glands to which they are carried, they are destroyed chiefly by phagocytic action. There is steadily increasing liberation of the bacterial toxins, and these, by local and general actions, cause active congestion of the invaded area, attraction of great numbers of polymorphs to it, and stimulation of the leucoblastic functions of the bone marrow. In the cases of the infected mucous membranes, the toxic action and congestion also stimulate the secretion of mucus and generally the pouring out of lymph and polymorphs on the surface. The bacterial attack may be completely overcome by these initial phagocytic and bacteriolytic actions ; large numbers of the polymorphs may die in the contest and form pus cells, or disintegrate. If the bacterial attack is not successfully overcome within a few days, the action of

various specific antibodies comes powerfully into play. In conjunction with the complement, they effect dissolution of many of the bacteria. If bacteriolytic action is in excess of antitoxin action, more toxin will be formed in the infected focus and there will be an increase of the local and general disturbances. The polymorphs and complement, in conjunction with these auxiliary forces, effect the destruction of the bacteria. As a result of the struggle, at least two changes are established within the body, both of a more or less temporary character, but lasting for some weeks, months, or even years: (1) specific antibacterial substances are retained in the body and they are readily increased in quantity by any fresh absorption of the corresponding toxin; and (2) a greatly increased tolerance of this toxin has been established.

State of the Bodily Defences in Chronic Infections

In chronic infections, a state of balance has been established between the attacking micro-organisms entrenched in the tissues and the anti-bacterial forces of the body. For example, in a chronic infection of the nasopharynx by *streptococcus pyogenes*, the streptococci are lying, not only at the surface of the mucous membrane, but also among the epithelial cells and subepithelial connective tissues. That this is so may readily be demonstrated by the microscopical examination of a swab from the infected area, taken with the exertion of considerable pressure, so that some of the epithelium is detached.

When a state of chronic infection of this kind is established, there are several things that may happen. Some marked improvement in the state of the general health of the patient, consequent, for example, upon taking a long rest in the country, if he is a town-dweller, may result in the anti-bacterial forces getting the upper hand and eradicating the bacteria. A much more common occurrence is an exacerbation of the infection, consequent upon the patient getting his body chilled. In some way, not yet exactly explained, chilling of the body, especially when combined with fatigue, is generally followed by aggravation of any existing local

chronic bacterial infection. Apparently there is for some hours active invasion by the bacteria from the already infected tissues into fresh neighbouring areas. The effects of this invasion are generally not felt until from twelve to twenty-four hours after the chilling occurred. Many explanations have been attempted, but the evidence for most of them is rather indefinite. It seems most probable that chilling of the body commonly results in diminution in the number of polymorphs in the blood, loss of their phagocytic power, and reduction in the amount of complement. This action of chills is often capable of revealing the fact that a person is suffering from some form of chronic infection. Attention is directed to the region by the local inflammation, bacteriological investigation reveals the nature of the infection, and a consideration of the history of the case may warrant the conclusion that this infection has been of long standing and that it is not one that has simply been recently acquired.

Such increased invasion from an infective focus implies liberation and absorption of a larger amount of bacterial toxin and a greater call upon the defensive forces. These may respond successfully, and beat back and destroy the invaders, or they may prove unequal to the task, and the citadel falls before the enemy. Apart from the sudden extension of the area of invasion from this cause, or aided by it, prolonged bacterial attack gradually wears out the bodily defences, impairs the functional action of vital organs, and shortens the term of life. No sleuth-hound ever followed his victim more ruthlessly than some of the common bacteria, such as the tubercle bacillus, *streptococcus pyogenes*, and pneumococci, press their attack. They may take years to accomplish what would almost seem to be their set purpose, but in the end, if some other enemy has not taken the task out of their hands, they slay. These facts give tremendous importance in practical medicine to the early suppression of chronic bacterial infections, and to therapeutic immunization as almost the only known means by which this purpose can be attained.

CHAPTER IV

THE THEORY OF THERAPEUTIC IMMUNIZATION

THERAPEUTIC immunization brings no new forces to the assistance of the body in its fight with its bacterial enemies. It merely strengthens already existing forces and turns the balance in favour of the host. Beyond all possibility of refutation, the fact has been established that, by introducing suitable quantities of the corresponding toxin into a healthy situation, pathogenic bacteria in an infective focus can be destroyed. It is equally certain that this result can be attained only if certain rigid conditions are fulfilled. The dose must be sufficient to stimulate the protective mechanisms strongly, and yet it must not be so large as to result in a distinct increase of the toxæmia already established; a stimulation of suitable intensity must be repeated frequently at proper intervals; the body must be in a state in which it is able to respond to the action of the toxins introduced; and the tissues at the seat of infection must not be so seriously damaged as to be incapable of survival.

It is therefore of the utmost importance that our procedure should be guided by definite principles founded upon ascertained laws of immunity, as well as upon actual experience gained in the practice of therapeutic immunization. To lay down these principles is the purpose of this chapter. Their practical application is considered in Chapter IX.

It must be admitted at the outset that we are not yet in a position to explain all that occurs in the body generally, and in the infective focus specially, when a dose of the corresponding bacterial toxin is introduced into a healthy area. Nevertheless, a satisfactory working hypothesis can be framed on the basis of established facts and of practical experience of the action of immunizing agents. We have passed a long way beyond the stage at which we can be said

to be still groping in the dark. Therapeutic immunization, rightly carried out, is already an instrument of the most potent kind, and it is certainly destined to undergo gradual extension of its application, until it takes a chief place in the practice of medicine.

The theory of therapeutic immunization that seems to me to accord best with the phenomena that can be observed may be briefly stated as follows :—If, in a person suffering from a local bacterial infection, a minute quantity of the corresponding bacterial toxin is injected into the healthy subcutaneous tissues, a powerful, but in large part transitory, stimulus is thereby given to the formation of specific antibodies. These accumulate in the blood, attaining the height of their development in from twelve to forty-eight hours, and then rapidly diminishing again. They reach the infective focus, where they combine with some of the most accessible bacteria, and, in conjunction with the complement, effect their dissolution. A result of this local destruction of bacteria is the liberation of endotoxin in the infective focus, which consequently becomes congested and invaded by polymorphs, which effect further destruction of bacteria. There may be overflow of the bacterial toxins into the general circulation, with consequent malaise and sometimes rise of temperature. If such focal reactions are repeated, at suitable intervals, by the same means, the bacteria in the infective focus may be entirely destroyed. At the same time, other more general effects are produced which result in a more enduring enhancement of the power to resist attack by the bacterium. Each dose of toxin injected is supplemented by a much larger one formed in the infective focus, and these together continue to stimulate (provided there is adequate power of response) the development of various antibodies, which tend to be retained, or to be formed afresh, for a long period, whilst also a great increase occurs in the tolerance of the toxin.

This theory of therapeutic immunization has many details that require further explanation. It may be more easily understood perhaps by consideration of what occurs when the method is applied in a case of simple chronic infection. Let us take as an example a chronic infection of the naso-

pharynx by a particular type of *streptococcus pyogenes*. A bacteriological examination has been made, a special strain of *streptococcus pyogenes* has been found to be present in very large numbers, and an immunizing emulsion of the strength 1 c.c. =0·1 mg. has been prepared. An initial dose of 0·2 c.c. [=·02 mg.] of this is injected under the skin of the back of the upper arm. Next day, at the point of injection there is nothing to be seen except, perhaps, very slight redness over a small area, and pain and tenderness are trivial. In the nasopharynx, however, there are distinct disturbances. The patient has sensations similar to those from which he is accustomed to suffer when his nasopharyngeal trouble is at its worst. There is much increase of mucous secretion, the back of the nose feels swollen, burning and even painful, and the nasal passages are more or less blocked. If the region is examined, it will be observed to be intensely congested. After some hours, these disturbances cease.

Let us endeavour to picture what has taken place. The injection of ·02 mg. of the dead streptococci under the skin of the arm was followed within a few hours by their more or less complete dissolution by phagocytic and bacteriolytic actions, and the liberation of some of their endotoxin. This endotoxin, whether acting merely locally or more generally is not clearly established, stimulated the formation of specific bacteriolytic antibodies. In the infective focus in the nasopharynx there were lying millions of living and dead streptococci, capable of being dissolved by specific bacteriolytic action. In about twelve hours the specific antibodies were so increased in the blood-stream as to make their influence felt in the infective focus. Becoming fixed to the protoplasm of the most accessible streptococci, they effected their dissolution with the aid of phagocytic and complemental actions. Consequent upon this dissolution of large numbers of the streptococci, there was a liberation of endotoxin, which immediately caused congestion of the area and favoured invasion by leucocytes. The area therefore became swollen and painful, and there was increased secretion of mucus and exudation of lymph. The wave of increased bacteriolytic action fell after some hours. Toxins passed into the lymphatics and general circulation, causing

some malaise, and were soon destroyed, or excreted. The infective focus returned to its previous condition, excepting that a considerable proportion of the streptococci lying within it were now destroyed.

If such a process as has just been pictured is repeated several times, the infecting bacteria may be completely eradicated. These bacteria are, however, continually multiplying, generally on the surface as well as in the subjacent tissues, and if nothing occurred in addition to what has just been described, the remedy would be only temporary and ineffectual. Important new factors are gradually introduced, as we repeat and enlarge the dose—namely, increased tolerance of toxin and increased power of resistance to the bacterial attack; the level of both slowly rises, and the high level reached is maintained for months or even years.

It is found that, in order to produce a similar focal reaction, the dose of dead bacteria has generally to be increased with each injection. There are at least two elements upon which this necessity of increasing the dose depends. First, the number of bacteria in the infective focus capable of being dissolved is gradually decreasing and a higher wave of specific bacteriolytic action is therefore each time required to dissolve a sufficient number to produce a focal reaction, and, after the bacteria are more or less completely eradicated from the infective focus, a focal reaction can no longer be induced. The second element necessitating a continual increase in the dose is one which, whatever its actual factors may be, expresses itself clearly in simple increase in tolerance for the bacterial toxin. This progressive increase in tolerance for the toxin is probably a response not merely to the toxin injected but also to the toxic wave that starts from the infective focus as the result of a focal reaction. Difficult as it may be to explain, the great practical fact remains clearly established by hundreds of observations that the power of resistance to the attack of a given bacterium is almost exactly proportional to the tolerance for the corresponding toxin. When, for example, a chronic infection by *streptococcus pyogenes* has been eradicated, a dose of the bacterial emulsion forty or fifty times greater than that

which at first caused severe malaise can be given without the patient suffering any disturbance. Part of this effect may be due to eradication of the infective focus, so that no additional toxin is absorbed from it. That this is not, however, an essential element is shown by the phenomena following protective inoculation, as, for example, against *bacillus influenzæ*, when there is no infective focus. An initial injection of, say, 0·04 mg., which causes well-marked malaise and drowsiness in most people in from twelve to twenty-four hours, may generally be repeated a few days afterwards without any disagreeable effects, but a dose of double the amount will generally cause similar symptoms to the first. With successive doses given at short intervals the tolerance for the toxin steadily rises, and in proportion to this tolerance is the patient's power of resistance to natural attack.

Tolerance of toxin is only one of several specific anti-bacterial factors capable of simultaneous increase. The others are numerous, and specific agglutinins, precipitins and bacteriolysins, the presence of which can generally be demonstrated, cannot rightly be regarded as representing all of them.

There are some additional points that still require consideration. If too small a dose of autogenous vaccine is given, the stimulation of the production of specific antibodies is too slight to make any impression upon the infective focus. There is no increased disintegration of the bacteria, and therefore no focal reaction. No benefit results. If a dose that must be regarded as excessive has been given, there is, first, a simple increase of the general toxæmia, and second, the development of a maximum wave of bacteriolytic action, with the result that there is a severe focal reaction, prolonged perhaps for several days. One stirring up, such as this, probably does good in the end, but experience proves that better results are obtained by a succession of focal reactions of moderate intensity. Not only does the production of a succession of intense and prolonged focal reactions cause the patient much suffering, but it may ultimately aggravate the malady, instead of ameliorating it. This harmful action of excessive doses is especially observable in therapeutic

immunization against the tubercle bacillus, the pneumococcus of rheumatoid arthritis, anaerobic diphtheroid bacilli in neurasthenia and exophthalmic goitre, and *staphylococcus pyogenes* in cases of recurrent boils. The toxæmia produced by large doses seems actually to favour the bacterial invasion.

In that class of infective diseases in which the chief pathological changes are produced at a distance from the infective focus by fixation of the toxins in particular tissues, the injection of doses of immunizing emulsion above a certain amount seriously aggravates the disease by increasing the toxæmia. To this class belong pneumococcal rheumatoid arthritis, the large group of neurasthenias dependent upon anaerobic diphtheroid bacillus infection, and dementia præcox and some other forms of mental disorder. All of these maladies can be aggravated at will by overdoses of the specific bacterial toxins. If we give overdoses of vaccine in the other class of infective diseases in which the characteristic pathological changes occur in the infective focus, we cannot but occasion toxic action in other parts of the body, although we have little definite knowledge of what they are. There are therefore strong grounds for the establishment of the rule that, in the practice of therapeutic immunization, the dose should not exceed one sufficient to produce a focal reaction of moderate intensity.

We should rely chiefly upon the elaboration of antibodies in the subcutaneous tissues; we should avoid increasing the general toxæmia. The patient has probably already achieved nearly all that is possible in the way of the production of antibodies as a response to the circulation of the toxin in the lymphatics leading from the infective focus and in the blood-stream. The subcutaneous tissues, the muscles, and some other organs and tissues, may remain uninfluenced by the toxins and retain the power of response when called upon to elaborate antibodies. In therapeutic immunization we are chiefly making a call upon certain reserve forces by introducing the toxin into a part of the body that it has not previously affected.

It has probably been more on account of the results of gross overdosage than from the use of wrong vaccines (common as this has been), that therapeutic immunization

has fallen into such disrepute among specialists and practitioners.

That it really makes a great difference whether the dose is of a certain amount, or ten times greater, may perhaps be impressed upon the reader if I record an illustrative experience. Some years ago I had occasion to make a bacterial investigation in the case of a doctor who was suffering from troublesome chronic nasal and post-nasal catarrh. I found evidence of severe infections by *micrococcus catarrhalis* and a diphtheroid bacillus. I prepared a vaccine of the strength 1 c.c. =*micrococcus catarrhalis*, 0·5 mg., and diphtheroid bacillus, 0·4 mg. I sent the vaccine to the doctor and directed that the initial dose should be 0·2 c.c. Instead of giving himself this amount, the patient injected 2 c.c., a dose of 1 mg. of *micrococcus catarrhalis* and 0·8 mg. of the diphtheroid bacillus. He reported by letter as follows :—"I was bad, very bad. Dose administered at 5.30 P.M. About 9.30 P.M. my arm felt so bad, and I felt so ill and chilly, that I went to bed at once and got a hot bottle at my feet and one at my back. I was too ill to take my pulse and temperature. To get warm was my great desire. I had a very restless night. I seemed to be among trenches and tanks the whole night. I had been to see the tanks in action the day before. Though feeling very seedy the day after, I took up my work as usual and gradually got better as the day advanced, though with a very painful and swollen arm."

A theoretical and practical question of great importance is whether there is any justification for the use of therapeutic immunization in acute bacterial infections, such as those by the pneumococcus, in a case of acute pneumonia, by *bacillus influenzæ* in acute influenza, *streptococcus pyogenes*, or *streptococcus anginosus*, in acute quinsy, and *micrococcus catarrhalis* in acute coryza.

The primary defensive action in acute infections is almost exclusively by means of phagocytic cells ; some days must elapse before specific bacteriolytic antibodies can be elaborated in sufficient quantity to play any important part in the defence. Are there any sound theoretical grounds upon which a corresponding vaccine may be used in such

circumstances? It has been denied that there are. Nevertheless, many observers have recorded good results from therapeutic immunization in acute infections. Others have pronounced it useless.

As an acute infection is generally overcome, not by phagocytic action alone (though in some cases it is), but by the gradual development and action of specific bacteriolytic antibodies, it seems to me rational to stimulate the formation of these antibodies. There are extensive areas of the body that are not affected by the bacterial toxins, and which therefore are not ordinarily called upon to elaborate antibodies. The subcutaneous tissues are among the areas that generally escape. By subcutaneous injection of the toxin we may therefore contribute to the formation of antibodies and supply the little more that often means so much. The chief danger will be the administration of doses so large that they seriously increase the general toxæmia. Therefore the dose must be small. It should be repeated every twenty-four hours; if possible, the vaccine should be a sensitized one. The vaccine must be one corresponding to the infection. As soon as possible, an autogenous vaccine should be substituted for the stock one with which treatment must be begun. These theoretical considerations have, as a matter of fact, now been amply confirmed by practical experience. It may be added in this connection that what is really desirable in acute infections is to be able to stimulate the production of antitoxins, rather than that of bacteriolytic antibodies. Perhaps some day it will be possible to prepare vaccines of a kind that will achieve this object.

The theory of therapeutic immunization here maintained may perhaps be more clearly understood from a few interpretations of some occurrences, such as are commonly observable in cases under treatment.

1. In cases of rheumatoid arthritis dependent upon infection of the gums and nasopharynx by a particular type of pneumococcus, doses of no more than 0·0005 mg. of an autogenous vaccine may be regularly followed in about forty-eight hours by an exacerbation of the pain in the joints, passing off in a few hours, or continuing for several days.

I believe the pain is due, not to liberation of pneumococcus

toxin previously fixed in the joint tissues, as has been suggested, but to liberation of toxin in the infective focus and conveyance of it to the joints by way of the blood-stream. The joint tissues have an affinity for the pneumococcus toxins. When the infective focus is eradicated, comparatively large doses of a pneumococcus vaccine can be given without causing any pain in the joints. So long as the infective focus is present, a minute dose of the vaccine involves liberation of very many times the amount of toxin contained in the emulsion injected. When the infective focus is eradicated, a very large dose of the vaccine will produce a general toxæmia, with pain in the affected joints. This pain is then due to the direct action of the toxin contained in the vaccine.

2. In a case of chronic infection of the respiratory tract by *bacillus influenzæ*, a first injection of the usual initial dose of the corresponding autogenous vaccine is followed by a violent attack of palpitation and cardiac weakness. The cases in which such symptoms occur are not very common, but they are encountered from time to time in the course of ordinary work in therapeutic immunization.

The cardiac disorder is not due to liberation of toxin previously fixed in the cardiac muscle, but is dependent upon liberation of influenza toxin in the infective focus and its passage into the blood-stream. Either the patient's heart muscle, or its intrinsic nervous apparatus, is highly sensitive to this special toxin.

3. A patient has had a long course of therapeutic immunization against *bacillus coli communis*, on account of chronic cystitis. On the day after a dose is given, some of the points at which previous injections were made "ache like the toothache."

Every hypodermic injection must leave a microscopic area of sclerosis, and, in this, some nerve twigs may be included; also some of the bacterial bodies injected may escape dissolution and remain in the sclerosed tissue. When subsequent injections of the vaccine are given, there is the usual wave of bacteriolytic action; the antibodies reach some of the bacilli remaining undissolved at these old sites of injection and cause their disintegration. A certain amount of toxin

is consequently liberated, and it affects the nerve twigs lying in immediate proximity, either directly or through local active congestion and pressure. Hence the spots are felt to ache. I believe that this is the true explanation of this phenomenon, so frequently experienced by patients who have been long under therapeutic immunization.

Hypersensitiveness to vaccines is, I believe, simply a consequence of the production of excessive focal reactions by vaccine doses, often pushed to ridiculous extremes, without accurate knowledge of the principles of dosage. Such hypersensitiveness, even to doses that must be considered minute, is certainly in some cases extreme. It has been interpreted by some as an anaphylactic phenomenon. This teaching has been very general and authoritative. In my judgment, it is quite erroneous, whilst being at the same time highly injurious to the interests of therapeutic immunization. Hypersensitiveness to vaccines needs no such hypothesis to explain it. Anaphylactic reactions and hypersensitiveness to vaccines differ radically from each other. They exhibit the following sharp contrasts:—

An anaphylactic reaction is a general reaction following quickly (within a few minutes) the injection of the protein to which the animal is sensitive. Hypersensitiveness to a bacterial vaccine does not show itself until from six to forty-eight hours after the injection has been given.

Anaphylaxis is not associated with any particular area of the body. Hypersensitiveness to a bacterial vaccine is essentially and chiefly manifested as a focal reaction at the seat of infection.

In anaphylaxis, the second injection must be many times greater than the original one. In hypersensitiveness to bacterial vaccines, the dose given is a mere fraction of the amount of the same toxin that is being absorbed from the infective focus every day.

Anaphylaxis is probably due to the formation of an antibody to a disintegration product of protein introduced parenterally and to its union with fresh antigen subsequently injected, the resulting product being a neurotoxic substance (Friedberger). Hypersensitiveness to bacterial vaccines does not permit of a similar explanation. The only view that

accords with the facts is that it is dependent upon disintegration of bacteria in an infective focus, with consequent liberation of toxin, through the stimulus to specific bacteriolytic action given by the vaccine.

An hypothesis may be advanced to explain extreme hypersensitiveness to bacterial vaccines. In some conditions of chronic infection, there is a ready responsiveness of the bacteriolytic functions to stimulation, as compared with the phagocytic and antitoxic functions; consequently, there is a liberation of a large amount of toxin at the seat of infection. This hypothesis is supported by the well-established fact that, when an infective focus is eradicated, hypersensitiveness disappears. When the area of bacterial infection is very extensive, the liability to hypersensitiveness appears to be increased.

There are some additional points of more than minor importance that require mention. Focal reactions may have a diagnostic value. Dermo-reactions, which have a different explanation, being more easily observed, have been chiefly studied. The occurrence of a focal reaction establishes the fact that the bacterium from which the vaccine was prepared is acting as a pathogenic agent in the case. In this way the special pathogenic actions of various common bacteria have been determined. When the observation of a series of these reactions is followed by recovery from the malady, we have established the pathogenic character of the bacterium by the method of focal reaction and therapeutic immunization.

A theoretical consideration may be advanced here. It seems clear that, in many forms of infective disease, bacterial toxins are liberated at an infective focus and carried to distant tissues that have a special affinity for them. These tissues become injured by the toxin, and special forms of disease arise. This explanation seems to hold for the joint disorder in rheumatoid arthritis of pneumococcus origin, the destruction of the bone marrow in pernicious anæmia, the injury to the cortical nerve cells in dementia præcox and acute confusional insanity, and the neurotoxic actions in the most common type of neurasthenia. The question arises whether we could not benefit the patient by intercepting these toxins by the introduction of suitable quantities

of the corresponding tissue element in solution. The action would be similar to that of an emulsion of the nervous tissues in neutralizing tetanus toxin. Joint-tissues might be used in this way in rheumatoid arthritis, cortical tissue in the toxic forms of insanity, bone marrow, or hæmoglobin, in pernicious anæmia.

A question of considerable practical importance is that of whether it is possible to produce a general stimulation, as distinguished from a specific one, of the defensive mechanisms by the injection of therapeutic doses of the toxins of common bacteria such as *staphylococcus pyogenes, streptococcus pyogenes, streptococcus fæcalis* and *bacillus coli communis.* There is some evidence in support of the view that this can be done. The action may be very similar to that of neuclein, which produces leucocytosis. There are grounds for believing, however, that the toxins, especially of *streptococcus fæcalis* and *bacillus coli communis*, have, apart altogether from their action as antigens, very definite and important pharmacological actions, beneficial to the human economy, which require systematic investigation.

An important question is that of the special value of sensitized vaccines. Sensitized vaccines were first suggested and used by Besredka in 1902. Sensitization consists in placing the vaccine in contact with the corresponding antiserum, from which it absorbs certain antibodies. It has been alleged that vaccines so prepared produce neither local nor focal reactions, that they immunize more quickly than non-sensitized vaccines, and that much larger doses can be given with proportionate increased benefit. As the preparation of sensitized vaccines requires the previous high immunization of some large animal to the corresponding bacterium, their employment is rather costly. This objection to their use would not, however, stand in the way of their general adoption if it was certain that they offer any decided advantage.

Thanks to the facilities so kindly afforded me by the Managers and Physician-Superintendents of the Royal Edinburgh Asylum, I have been enabled, continuously since 1902, to keep a flock of sheep for experimental purposes, and for a long period they were used chiefly for the production

of specific anti-sera for the preparation of sensitized vaccines. An immune serum was produced for each of the common pathogenic bacteria, and for several years vaccines were systematically sensitized and used by myself and others. I need do no more than simply summarize the conclusions I have formed from these observations.

Most of the special claims that have been made for sensitized vaccines are justified exclusively, and with considerable limitation, as regards their action in acute infections. They do not hold for chronic infections to any important extent. In these they may act more rapidly, and slightly larger doses may be tolerated, but it is not true that they do not produce focal reactions. Indeed, if they did not produce focal reactions, it is difficult to understand how they could benefit the patient. It is true, as has been alleged, that they do not produce focal reactions in acute infections, but those who have put forward this claim as an advantage misunderstand the phenomena attending therapeutic immunization in acute as compared with chronic infections. In the former, the infective foci are already intensely congested, and focal reactions, if they occur in them, are not perceptible. What has been confused with focal reactions in acute cases is simple increase of the general toxæmia. With these qualifications, the fact remains that it is in acute infections that sensitized vaccines really have practical advantages. In these they act more quickly and more effectively, and comparatively large doses may be given without serious increase of the general toxæmia.

The theoretical grounds on which these advantages may be explained may be stated thus. The dead bacteria contained in a non-sensitized vaccine are insoluble, or only very slowly soluble, unless they are acted upon by specific antibodies in conjunction with complement, and they must be dissolved in order that their toxins may be liberated and made available for the stimulation of the specific defensive mechanism. Specific antibodies are present in considerable amount in the blood of every patient suffering from a chronic bacterial infection, but they may be absent, or present only in very small amount, when the infection is acute and of recent origin. In cases of acute infection, therefore, there

may be delay, or even failure, in the action of a vaccine, because of the lack of specific antibodies to combine with it. If the bacteria are saturated with antibodies before they are injected, their dissolution will occur under the action of complement with the maximum of rapidity, and there will be a corresponding acceleration of the defensive action. Hence, it is of value to use a sensitized vaccine in cases of acute infection, and hardly worth while doing so in chronic infections.

There is still one other question that remains to be considered—namely, that of the importance to be attached to detoxicated vaccines, which have lately been put on the market, prominently advertised and alleged to be superior to all other forms of vaccine. They have been described and advocated by Dr David Thomson (*The Lancet*, 8th March and 28th June 1919). They are prepared by dissolving the bacteria in normal caustic soda solution and reprecipitating them with normal hydrochloric acid. The precipitate is used as the vaccine, and it is alleged that the endotoxin has been removed by the process. This method, originally applied to therapeutic immunization against acute infections by the gonococcus, has been advocated as one of general application and as calculated to " revolutionize the whole subject of vaccine treatment and preventive inoculation." In support of his contentions, Dr Thomson has made some statements that are not in accord with facts observed by others. He makes it appear that we are troubled in therapeutic immunization by toxicity of vaccines, on account of which we have to limit the dose. In chronic infections at least, in which therapeutic immunization has its chief application, this is not the case. It is the intensity of the focal reaction that limits the dose, and not the toxicity of the vaccine. In acute infections, different principles apply, and it may quite well be that for these, the process described by Dr Thomson produces a vaccine that can be tolerated in comparatively large doses and that will give better immunizing effects. Even here, however, there is unfortunate vagueness. The detoxicated vaccine is standardized as an undetoxicated vaccine in millions. It has been put through a process of solution and precipitation,

in which something is lost. How much is not stated. An estimation made by Mr J. J. Ritchie with an emulsion of *micrococcus catarrhalis* showed that 50% of the weight was lost. It seems probable that with an organism like the gonococcus, which is more easily dissolved, the loss is greater. In any case, it is very misleading to standardize a "detoxicated vaccine" in terms of an ordinary one, and to compare the doses of the two as if the figures represented corresponding quantities.

It seems to me that one of the chief lines of advance in therapeutic immunization will be concerned with the methods of preparation of the immunizing emulsions, the composition of which will thereby be varied to suit particular purposes. It is extremely unlikely, however, that any one method will be found of general application. The wide claims already made for detoxicated vaccines cannot be regarded as established, and anyone with experience of therapeutic immunization must feel that they have been made the subject of too hasty generalization.

It would appear from the evidence of several observers, among whom is to be specially mentioned Dr David Lees (*The Lancet*, 28th June 1919), that "detoxicated vaccines" have an advantage over ordinary vaccines in the treatment of acute gonococcus infections. It must be said, however, that very good results can also generally be obtained with ordinary vaccines, if the dose is boldly pushed up in the course of five or six injections from 0·1 mg. to 1 mg. It is to be admitted that this is done at the cost to the patient of much general malaise and headache. If these symptoms are always trivial, even in cases in which "detoxicated vaccines" are pushed to their extreme doses, the observers no doubt have the patient's word for it, but some scepticism may be permissible. It is quite possible that, in these cases, "detoxicated vaccines" have a genuine advantage which they may also have in other acute infections, although in what it consists has not been shown. While "detoxicated vaccines" may have advantages in some acute infections, it is almost incredible that they can have any in chronic infections. In these, vaccines can act beneficially only by destroying bacteria in an infective focus. This involves

liberation of endotoxin. If a "detoxicated vaccine" does not do this, it cannot be beneficial; if it does, then the amount of toxin liberated is many times greater than even the minute quantity of the ordinary vaccine required to achieve the same purpose. Therefore it becomes of no moment whether the vaccine is "detoxicated" or not.

I am equally sceptical of the applicability of "detoxicated vaccines" to protective inoculation. The degree of protection is exactly proportionate to tolerance of toxin, and if the toxin has been eliminated from the vaccine, as has been alleged, it is difficult to understand how there can be any protection at all.

Such is the theory of therapeutic immunization. Before we are in a position to carry these theoretical considerations into practice, we must know much regarding the cultural characters and actions of the chief pathogenic bacteria, their clinical investigation and the method of preparing immunizing emulsions. Four sections of this book are devoted to these subjects, and it would probably be advisable for some readers to pass at once to a preliminary perusal of Chapter IX.

CHAPTER V

BACTERIOLOGICAL METHODS

Apparatus. Preparation of Culture and Test Media. The Making and Study of Cultures

It is intended in this chapter to give an account only of the methods essential for the limited class of bacteriological investigations required as a preliminary to therapeutic immunization. For other classes of bacteriological work, much additional apparatus and many other methods of investigation are necessary. These are described in the text-books of systematic bacteriology.

A few words may be said, first, about the accommodation required for the laboratory. A suite of rooms, luxuriously equipped, is not really essential for good work. A single large apartment will suffice, if it is well lighted and provided with a few necessary fittings. It is, however, better to have several smaller rooms, each devoted to its special purpose, such as the preparation of media, the storage of apparatus and reagents, cultural work, etc. Among the necessaries are water and gas supplies; two, or more, large tables, preferably placed near the centre of the room; and two, or more, large cupboards for storage purposes. If there is an electrical supply, as is extremely desirable, there should be a strong overhead light and a lamp with ground glass, placed about two inches above the table for illuminating the microscope. There must be gas pipes carried to suitable places for the heating of the sterilizers and incubators.

The following is a fairly comprehensive list of the requisite apparatus and reagents:—

Autoclave.
Koch's steam sterilizer.
Hot air, or dry sterilizer.
Incubator, to work at 37° C.
Microscope, with low-power and oil-immersion lenses.

Geryk vacuum pump.
Centrifuge.
Scales for weighing grams and ounces.
Chemical balance.
Bunsen burners.
India-rubber gas tubing.
Porcelain basins. The most useful sizes are 3, 6 and 8 ounces.
Beakers.
Glass flasks, 100 c.c., 500 c.c., 1000 c.c.
Burettes.
Glass funnels.
Glass measures, 100 c.c., 500 c.c., 1000 c.c.
Test tubes, 4 × ½ in., 5 × ⅝ in., 6 × 1 in.
Thicker glass tubes, 6 × ½ in., for swabs.
Specimen tubes, 3 × ¾ in.
Iron Wire. B.W.G. 17, galvanized.
Adjustable lenses for the study of cultures in ⅝-in. diameter tubes. (These lenses can be obtained from Messrs Melville & Hunter, 15 Bristo Street, Edinburgh.)
Grease pencils, red, blue, yellow.
Stand for holding microscopic slide in oblique position.
Platinum wire.
Glass rods.
Glass tubing.
Glass urine jars.
Microscopical slides, 3 × 1 in.
Cover glasses, No. 1.
Gummed labels.
Filter papers, 9 c.m and 32 c.m.
Aniline dyes: gentian violet, methylene blue, basic fuchsine, neutral red.
Albumen peptone.
Lemco.
Agar.
Gelatine.
Lactose, glucose, saccharose, mannite, salicin, inulin, maltose, galactose, raffinose, dextrine.

A supply of the following should always be at hand :—

1. Test tubes, 5 × ⅝ in., cleaned and dried, plugged with cotton wool and sterilized in the hot air sterilizer.
2. Test tubes, 4 × ½ in., cleaned, plugged and sterilized.
3. Test tubes, 6 × 1 in., cleaned, plugged and sterilized.
4. Glass flasks, cleaned, plugged and sterilized.
5. Medium size and large test tubes filled with distilled water, sterilized in autoclave.
6. Medium size and large test tubes filled with ¾% salt solution, sterilized in autoclave.

7. 100 c.c. flasks containing ¾% salt solution, sterilized.

8. Swab tubes. These are best made with fairly thick glass tubes of the size 6 × ½ in. For each, a well-fitting cork and a galvanized iron wire (B.W.G. 17) of suitable length are required. With the aid of pliers, one end of the wire should be bent into a round or square loop to form a handle. The other end must be burred by pressure in a vice, so that it may give a proper grip to the cotton wool that is to be rolled round it. Pass the wire through the centre of the cork, from its outer to its inner end, and wrap firmly round the point a sufficient amount of cotton wool. Place the wire thus prepared within the tube, fix the cork firmly in the mouth of the tube, and sterilize by dry heat.

9. Swab immersion tubes. Pour into sterilized swab tubes ordinary nutrient agar to a depth of about one inch, plug with cotton wool and sterilize in the autoclave. Allow the agar to set. Take each plug out, dip it in melted hard paraffin and immediately replace it. The object of this procedure is to prevent evaporation. Swab immersion tubes are very useful when swab specimens have to be sent from a distance. After a specimen has been taken, instead of the swab being replaced in its own tube, in which it would soon dry up, it is plunged deep into the agar in one of these immersion tubes. The specimen in thus kept moist until it reaches the laboratory. Many delicate pathogenic bacteria are killed by drying.

10. Specimen tubes, corked and sterilized in the dry sterilizer.

11. Stool specimen tubes. These are made by pushing strong iron wire through the cork of a 3 × ¾ in. sample tube and then forming a hook at the inner end. The cork, with the wire thus attached, is fixed in the tube. Sterilization must be carried out by dry heat.

Some additional apparatus, required in the preparation of vaccines, is detailed in Chapter VIII.

Preparation of Solid Media, Nutrient Broths and Test Broths

1. Nutrient Agar.

Lemco	10 grams (1%)
Albumen peptone	10 grams (1%)
Sodium chloride	5 grams (½%)
Agar	20 grams (2%)
Water	1000 c.c.

Put these in a glass flask and plug the vessel with cotton wool. Place in the autoclave and keep at temperature of 105° C. for forty-five minutes. Make the medium slightly alkaline by adding 5% sodium hydrate solution, until it turns red litmus slightly blue (it should not turn blue litmus

red). Filter the hot fluid through filter paper in the Koch sterilizer at 100° C. Place a glass cover over the filter funnel in order to prevent condensation water dropping into the medium. Fill the medium into previously plugged and sterilized tubes (5 × $\frac{5}{8}$ in.) to one-fifth of their depth. Place the tubes in the autoclave for one hour at 105° C., and then slope them on a suitable support, taking care that the medium does not touch the cotton-wool plugs.

For most purposes the above simple method of obtaining the right reaction will suffice, but if it is desired to provide a medium of a definite degree of acidity or alkalinity, the exact degree must be ascertained and corrected as required.

A medium of a standard acidity or alkalinity is prepared by adding so many cubic centimetres of a normal acid or normal alkaline solution to a litre of the neutral medium. The sign + is commonly used to denote acidity and the sign − to denote alkalinity. A normal solution is one containing an equivalent (the weight in grams chemically equivalent to one gram of hydrogen) of the substance in a litre of water. For example, normal solution of sodium hydrate contains 40·06 grams per litre. Normal solution of sulphuric acid contains one half of the molecular weight, because the acid is dibasic. Equal volumes of a normal acid solution and of a normal alkali solution exactly neutralize each other.

Normal caustic soda solution, with which we have chiefly to deal here, is prepared, as already indicated, by adding 40·06 grams pure sodium hydroxide to one litre of water. It is generally kept in a bottle with a rubber stopper, through which there pass two glass tubes. One of these, carried down on the outside of the bottle to the level of the base and fitted with a rubber tube and clip, serves for drawing off the fluid; the other is provided with a bulb filled with dry soda lime to absorb carbonic acid gas contained in the air that enters by it to replace fluid drawn off.

Normal hydrochloric acid contains 36·5 grams of hydrochloric acid per litre. It is prepared by titration against normal caustic soda.

To prepare +15 nutrient agar, proceed as above directed, as far as the first sterilization. Measure out 20 c.c. of the hot medium and place it in a beaker. Measure out the same

quantity in another beaker. Add 20 c.c. of water to each and boil over a Bunsen flame to remove carbonic acid gas, as the indicator used does not act if this is present. To the first beaker only, add a few drops of a 0·5% solution of phenolphthalein in neutral methylated spirit. The purpose of the second beaker is simply that of a control by which the earliest indication of a pink tint in the first may be detected. Fill a burette with a 1 in 20 dilution of normal sodium hydrate $\left(\frac{N}{20}\ NaOH\right)$. Run this solution into the agar in the first beaker until a faint pink colour appears in it. Estimate the degree of acidity from the amount of the reagent used. Thus, if 20 c.c. of the agar medium required 8·5 c.c. of $\frac{N}{20}$ NaOH for neutralization, 1000 c.c. will need 425 c.c. of this dilute solution, or 21·25 c.c. of Normal NaOH. The medium is thus +21·25. To reduce this to +15, there must be added 6·25 c.c. of normal sodium hydrate solution to 1000 c.c. of the medium. But the volume of the medium is now 960 c.c. and therefore only 6 c.c. are required. Shake the medium and test it again. If the reaction is now correct we should find that 6 c.c. of $\frac{N}{20}$ NaOH are required to neutralize 20 c.c. To neutralize 1000 c.c., there would therefore be required 300 c.c. of this dilute solution, or 15 c.c. of normal sodium hydrate, and the medium has thus the reaction +15.

To make a + 30 agar, add the number of cubic centimetres of normal hydrochloric acid solution required to bring the reaction to this point. In the above instance, the amount required for 1000 c.c. is 8·75 c.c., and for 960 c.c., 8·4 c.c. Complete the preparation of the medium by filling it into tubes, and sterilizing and sloping, as already directed.

The following account of an actual example of the steps taken in making up a batch of +30 agar may help to make the procedure clearer. In this instance the control beaker of medium was dispensed with, as being unnecessary after a little experience has been gained. A flask containing 500 c.c. of nutrient agar had been sterilized in the autoclave. While it was still hot, 20 c.c. of the medium were measured out and placed in a beaker. About an equal quantity of water

was added. The beaker was then placed on an asbestos sheet lying on the top of an iron stand, and a Bunsen flame was placed below. A 1 in 20 dilution of normal caustic soda was next made up. A burette graduated from 1 c.c. to 100 c.c. was washed out with a little of this solution, fixed vertically in its stand, and then filled with the same solution. This was run out a little until it stood exactly at the zero mark, care being taken also that no air bubbles remained below the tap. The medium in the beaker was now boiling. The beaker was placed below the burette and a few drops of phenolphthalein solution were added to it. The tap of the burette was slightly opened and the caustic soda solution dropped slowly into the hot medium, which was kept in motion by being stirred with a glass rod. After a little, a faint pink tint began to appear temporarily throughout the fluid. The tap was immediately turned off and the amount of caustic soda that had been used was noted. A few more drops were allowed to fall from the burette, until a faint pink tint became permanent. The amount of caustic soda used was again noted. The mean between the first and the second readings was taken as the point of neutralization. The figure was 7·9 c.c. If this quantity of caustic soda solution was required to neutralize 20 c.c. of the medium, 395 c.c. would be required to neutralize one litre of it. But as the caustic soda solution was diluted to 1 in 20, only 19·75 c.c. of normal caustic soda would be required. The medium was therefore +19·75. To bring one litre of it up to the required +30 strength, 10·25 c.c. of normal hydrochloric acid would have to be added. But there were only 480 c.c. of the medium, so only 4·9 c.c. were needed. This quantity of normal hydrochloric acid was therefore measured out and added to the medium remaining in the flask.

The Lemco and water in the above medium may be replaced by 1000 c.c. of meat extract, prepared in the following way :—Take 1 lb. of finely minced flesh (horse, ox or calf), free from fat, and add to it 1000 c.c. of distilled water. Mix thoroughly in a shallow dish and set aside in a cool place for twenty-four hours. Skim off any fat, removing the last traces of it by stroking the surface of the liquid with pieces of filter paper. Place a clean linen cloth over the mouth of

a large filter funnel and strain the liquid through it into a glass flask. Take the cloth containing the meat and squeeze out any remaining juice, finishing the process by placing the cloth and its contents in a meat press. Boil the filtrate for two hours and then strain it through a clean cloth. Repeat the boiling for two hours and filter through filter paper. Make up the volume to 1000 c.c. with distilled water. The resulting fluid should be quite transparent. If it shows any redness, it must be boiled again and filtered. Finally sterilize in the autoclave for forty-five minutes at 105° C.

2. **Lactose Agar.**—This is nutrient agar with the addition of 1% lactose.

3. **Hæmoglobin Agar.**

Lemco	10 grams (1%)
Lactose	10 grams (1%)
Albumen peptone	10 grams (1%)
Sodium chloride	5 grams (½%)
Agar	30 grams (3%)
Water	1000 c.c.

Prepare in the same way as nutrient agar, but filter through a jelly bag instead of filter paper, as a perfectly clear medium is not necessary. After the tubes have been sterilized in the autoclave, place them in a vertical position and allow to cool to a little below 60° C. This temperature can be estimated with sufficient accuracy by testing a tube with the hand, to which the heat should be just comfortable and not painful. While the medium is at about this temperature, with the usual aseptic precautions, pour into each tube a few drops of hæmoglobin serum, immediately rotating the tube in the hand until the medium is of a uniform red colour. Slope the tube on a suitable support and allow the medium to set. It is important that the medium should be of a deep red colour. It is of little use for the differentiation of colonies if the percentage of hæmoglobin in it is too low.

Hæmoglobin serum is obtained from the blood of the sheep or ox, collected with aseptic precautions. After coagulation has taken place (the clotted blood may be kept in the tubes for many days), put the tube in a mixture of ice and salt (2 to 1) until blood is frozen, and then in warm

water. The serum soon acquires a rich red colour ; it should then be decanted into sterile tubes and tested for sterility.

Hæmoglobin agar was introduced in 1902 by Dr Douglas McRae and myself. It has since been used constantly in the Laboratory of the Scottish Asylums.

Special Uses of Hæmoglobin Agar.—This medium is essential for the bacteriological investigation of cases. Not only is the growth of the commoner bacteria generally much more vigorous upon it than upon ordinary agar, but many additional characters, serviceable for identification, are manifested. Thus, certain species of streptococcus form rocky whilst others form soft colonies. Hæmolytic action (strictly hæmoglobinolytic) is clearly shown and has considerable importance as a means of identification of species. Moreover, the hæmolytic change has various special features. It may spread from the colonies, it may be confined to the area below the colony, it may be diffused through the medium, or it may display itself in a mustard coloration. Some bacteria grown upon this medium produce a curious grey fog beyond the margin of the growth, whilst underneath the colonies the normal appearance persists. Hæmoglobin agar serves for the growth of various bacteria that are regarded as difficult to grow, and for which other special media are commonly thought to be necessary, including pneumococci, bacillus of influenza, Bordet-Gengou bacillus and the gonococcus. It is also of great service in its application to anaerobic methods.

Variations in the Reaction of Hæmoglobin Agar.—The most useful medium is one that is alkaline to litmus, but acid to phenolphthalein to the extent of 15 on the Eyre scale. Higher degrees of acidity are required for special purposes. Thus the *bacillus acnes* requires about 30. On the other hand, there are purposes for which a greater alkalinity is necessary. Directions for the preparation of the nutrient agar basis of a medium of such definite degrees of acidity have already been given. It must be borne in mind that 3% agar, and not 2%, is required for hæmoglobin media.

Hæmolytic action is shown better on a +7·5 medium than upon a +15 one. Hæmolysis does not occur in a +30 hæmoglobin agar. The three media at present in use at

the Laboratory of the Scottish Asylums are +6 (alkaline), +18 (neutral) and +30 (acid).

4. **Blood Agar.**—This may be prepared in one or other of two ways. The first is to smear the surface of an agar medium with sterile human blood, generally obtained from the finger, and carried from the finger to the agar surface in a large platinum loop.

The following method of procuring the blood will be found to have advantages :—sterilize the skin on the back of the terminal phalanx of a thumb or finger with 5% carbolic acid in alcohol. Wind a piece of string somewhat tightly round the base of the thumb or finger, and wash the sterilized surface with absolute alcohol. Allow this to evaporate and then spread over the surface with a glass rod a drop of warm vaseline, previously sterilized by heat in a test tube and allowed to cool. With a sterile needle, prick the skin through the layer of vaseline. The blood rises to the surface and can then be rapidly transferred with a platinum loop to several agar tubes.

The other method is to collect ten or twelve drops of fresh blood in a tube containing about 10 c.c. of 1% sodium citrate solution (previously sterilized), and to add about a half centimetre of this diluted blood to each of several tubes containing melted agar medium, as in the preparation of hæmoglobin agar. If, in obtaining the blood in the manner described above, the thumb is forcibly flexed, sufficient blood to make from twelve to twenty blood agar tubes of this kind may generally be obtained in a few seconds. The blood, as it flows, should be made to drop into the tube containing the sodium citrate solution.

5. **Dorset's Egg Medium** (see Gordon (9), p. 97).—Wash two or three fresh eggs. Sterilize the shells by immersion in pure formalin. Allow the shells to dry. Break the eggs over a sterilized dish. Mix yolk and white thoroughly by stirring. Strain through sterilized gauze. Measure the volume and add 0·85% salt solution (sterilized) in proportion of 1 to 3 parts of egg. Glycerine may be added by using, in place of the above salt solution, 0·6% solution of glycerine in 0·85% salt. It is of advantage also to add a few drops of alcoholic solution of basic fuchsine as a colouring matter.

Decant the mixture in suitable amount into sterilized test tubes and solidify it in the sloping position at a temperature of 70° C., maintained for four hours.

6. **Nutrient Gelatine.**

Lemco	4 grams (1%)
Albumen peptone . .	4 grams (1%)
Sodium chloride . . .	2 grams (½%)
Gelatine, in summer . .	60 grams (15%)
in winter . .	40 grams (10%)
Water	400 c.c.

Dissolve these ingredients by placing the flask containing them in the steamer at 100° C. for twenty minutes. Render the medium slightly alkaline to litmus. Filter through paper in steam sterilizer. If the medium is still opaque, add white of egg, boil for ten minutes, and filter, afterwards making up to original volume. Fill into tubes and sterilize in steam sterilizer for twenty minutes on three successive days. Allow to solidify with tubes in erect position, not sloped.

Nutrient gelatine is required for the identification of species in the coli-typhoid and staphylococcus groups, Stab cultures are made to a depth of about half-an-inch. Incubation must be carried out at a temperature of about 22° C. This temperature can generally be obtained under a bell-jar placed on the top of the incubator working at 37° C.

7. **Lemco-Peptone Broth.**

Lemco	10 grams (1%)
Albumen peptone . .	10 grams (1%)
Sodium chloride . . .	5 grams (½%)
Water	1000 c.c.

Sterilize in autoclave for three-quarters of an hour at 105° C. Make slightly alkaline with 5% caustic soda solution. Filter through paper when cold and fill into tubes. Sterilize tubes in autoclave at 105° C. for three-quarters of an hour. The reaction should be from +18 to +20 on the Eyre scale.

8. **Lemco-Lactose Peptone Broth.**—This is prepared in the same way as Lemco-peptone broth, with the addition of 1% lactose.

9. **Serum Broth.**—This is prepared by adding sheep's

serum (sterile) to Lemco lactose peptone broth, in the proportion of 1 to 9. It is important that a higher proportion of serum be not added. The tubes should be incubated before use, in order to test the sterility of the broth. In practice it is found most convenient to store serum broth in 10 c.c. quantities in 5 $\times\frac{5}{8}$ in. tubes, and to decant into 4 $\times\frac{1}{2}$ in. tubes immediately before use.

The important use of this serum is that of showing the length of the chains of a streptococcus. The same cultures may subsequently serve for the purpose of vaccine preparation.

10. **Peptone Water.**

Albumen peptone . .	5 grams (1%)
Sodium chloride . . .	2·5 grams ($\frac{1}{2}$%)
Distilled water . . .	500 c.c.

Place in steamer at 100° C. for half-an-hour. Allow to cool and filter through paper. Fill into tubes, putting 10 c.c. in each. Sterilize in autoclave for half-an-hour at 115° C.

Peptone water is required for the indol test in the differentiation of the members of the coli-typhoid and proteus groups.

11. **Glucose-Peptone Broth.**

Albumen peptone . .	5 grams (1%)
Glucose	5 grams (1%)
Sodium chloride . . .	2·5 grams ($\frac{1}{2}$%)
Water	500 c.c.

Prepare further as directed for peptone water.

This broth is used in the Voges and Proskauer test for the differentiation of certain members of the coli-typhoid group.

12. **Hiss's Serum Water Media.**—These are composed of one part of serum (of sheep or ox) and three parts of distilled water, with 1% litmus and 1% of a pure sugar, or other test agent, added. They may conveniently be prepared as follows:—Add 4 grams lactose (or other test agent) to 150 c.c. sterilized distilled water. Add 4 grams litmus to 150° c.c. sterilized distilled water. Boil each of these solutions in separate sterilized flasks for half-an-hour at 100° C. Filter both into one large sterilized flask and add 100 c.c. sterile serum. Mix by shaking. Fill into sterilized tubes (4 $\times\frac{1}{2}$ in.). Further sterilize the filled tubes for an hour at 100° C.

The cotton-wool of the plugs should be dyed with Drummer

dyes, each colour being made to indicate a particular test agent. These broths serve in an important way for the differentiation of species included in certain of the bacterial groups. The reaction depends upon the formation of acid from the test agent by the vital action of the bacterium; this is shown first by red coloration, and afterwards, if sufficient acid is formed, by clotting of the medium. It is essential for ordinary bacteriological work to have the following test broths: — lactose, glucose, saccharose, salicin, mannite, raffinose, inulin, galactose, maltose and dextrin.

13. **Litmus Milk.**—Place a suitable quantity of skimmed milk in a flask and heat to 100° C. for half-an-hour. Put in a cool place overnight. Siphon off the milk, leaving the cream. Add enough watery solution of litmus to colour. Fill into small test tubes and sterilize at 100° C., for twenty minutes on three successive days.

Litmus milk is used for the differentiation especially of members of the coli-typhoid and proteus groups, some of which cause an acid change in it, revealed by clotting and reddening.

14. **Acid Urea Broth.**—Take

Lemco	4 grams (1%)
Albumen peptone . .	4 grams (1%)
Water	400 c.c.

Keep these for half-an-hour at 100° C., allow to cool, filter through paper, and add

Urea	8 grams (2%)
Neutral red ½% solution in water (freshly prepared)	2 c.c

Mix and dissolve. Render faintly acid with hydrochloric acid. Acidity is indicated by a pink tint of the fluid, alkalinity by a yellow colour. Put a little of the broth into two tubes and keep these at 100° C. for one hour. Examine to ascertain if the pink tint remains. If it does not, add more acid. When the reaction has been ascertained to be correct, fill the fluid into small test tubes and sterilize for one hour at 100° C.

Acid urea broths yield a reaction that helps to differentiate members of the coli-typhoid, proteus and other groups.

Some bacteria are able to convert the urea into ammonium carbonate, the presence of which is indicated by a yellow coloration of the fluid.

Method of Analysing a Bacterial Flora

As a preliminary to the correct application of therapeutic immunization by means of autogenous vaccines, it is necessary to make an analysis of the bacterial flora of the seat of infection. For this purpose, we secure suitable material from the infected region and make cultures in the way that experience has shown to be the most likely to be successful. When colonies have developed, we proceed to make sub-cultures of the various types that are present, with a view subsequently to identifying the species by ascertaining their microscopical, biochemical and other characters. In order to get pure growths it is still the fashion to use the method of plating out in Petri dishes. I advise that this method should be discarded in bacteriological work of the kind that is required for therapeutic immunization. I gave up plating many years ago, because I learned from experience that there are much better methods of analysing a bacterial flora. There is nothing absolutely new in the routine method about to be described, although its success depends upon the use, first, of hæmoglobin agar, and second, of the lenses attachable to the culture tubes, devised by Mr J. J Ritchie.

The obtaining of culture material and the making of cultures has to be adapted to various special requirements depending upon the location of the infection, the nature of the material containing the bacteria and the expected character of the growth. So important and many-sided is this matter that a whole chapter (VII.) is devoted to it. What it is necessary to give here is a simple general outline of the routine to be employed. The standard medium is hæmoglobin agar (+15 to +18), sloped in a tube of the size $5 \times \frac{5}{8}$ in. Simple nutrient agar tubes are used chiefly for breaking up a culture material, preparatory to implanting it upon a hæmoglobin surface. With attention to any special measures that the case may require, as explained in

Chapter VII., the material is spread on the surface of several tubes with a platinum loop in various dilutions. We wish to secure, as far as possible, that the colonies will be numerous and yet separate from each other. After the tubes have been inoculated, the cultures may either be treated as aerobic or put under more or less strict anaerobic conditions. If aerobic conditions are to be given to the cultures, they are simply placed in the incubator at 37° C. overnight. Next day, the surface will be studded with more or less distinct colonies, probably of various types. We wish to recognize and transfer to other tubes specimens of each type present. The growths in each tube must be carefully examined under a low-power adjustable lens, which can be moved along the tube and focused on the surface of the medium. It may be necessary to warm a tube slightly over the Bunsen flame, opposite the agar surface, in order to dry off moisture on the inside which obscures the view. Make notes in the record book of the features of the growth in each primary culture. Suppose that three different types of colony have been recognized. Make a four or five drill subculture of each type in succession on tubes of medium of the same kind. For this purpose remove the cotton-wool plug from the tube containing the primary culture, and hold it and the tube in the left hand. While the colonies to be subcultured are kept in focus under the adjustable lens, touch a selected one with a small platinum loop or point, previously sterilized in the Bunsen flame and allowed to cool, being careful to make sure that a portion of the colony actually adheres to it. Withdraw the wire without touching any other growths, the medium, or the glass, and stroke it gently over the agar surface in a fresh tube from the lower end, just above the water of condensation, to the top. The first stroke should be made on the left side of the surface, the left edge of the medium being regarded as that which is on the left when the tube is held in front of you, in an upright position with the surface turned away. From four to six separate evenly spaced strokes derived from the same type of colony can generally be made upon one surface. Fix a gummed label on the glass and write on it the name, "Subculture 1," and the date. Make similar subcultures in successive tubes of

as many types of colony as are represented in the primary growths. It is not necessary to make a similar analysis of the colonies in each primary growth. What we require is merely a series of subcultures representative of every type of colony that is present. The subcultures must immediately be placed in the incubator ; the primary growths should be carefully set aside in a dark cupboard, as it may be necessary to examine them again.

As a rule, after twenty-four hours' incubation the subcultures have sufficiently developed to permit of examination. Inspect each in turn with the aid of the adjustable lens. First note and record the characters of each growth in Subculture 1. Each growth in this tube must next be examined microscopically. Take a 3 × 1 in. microscopical glass slide, clean it with a towel, and pass one side of it several times over, or through, the Bunsen flame until the glass is very hot. Have at hand red and blue grease pencils. Holding the heated slide by one end with burnt surface uppermost, mark on this surface, near the right edge, with the pencils, as many short lines as there are growths in the tube. The first mark should be red, the second blue, the third red, and so on. Thus the odd numbers will be red and the even ones blue. Now lay the slide on an oblique slide support and place it in such a way that a bright reflection can be obtained from it. With a sterile platinum loop, or point, next carry to the glass, just beyond the point of the mark No. 1, a little of the culture from the first growth in the tube. Sterilize the platinum needle and similarly transfer a minute quantity of growth from the second stroke to the glass just beyond the point of mark No. 2. Deal with the remaining growths in a similar way. Fix and stain by the Gram-neutral red method, as directed under staining methods for films. Examine the specimen first under the low-power and then under the oil-immersion lens, using the red and blue marks as guides to the successive films. Record the appearances. Note whether the organisms are Gram-fast or Gram-negative, their shape and size, and if the growth appears to be a pure or a mixed one. If the growths are pure, it will generally be possible to give each a name and number, which, however, need not indicate a final

identification of species. Thus, if all the growths in the first tube are of the same kind, they may be designated "staphylococcus," "streptococcus," "Gram-negative diplococcus," "coliform bacillus," etc., and numbered from 1 onwards. If the identification is not complete, it will be necessary to proceed to make such subcultures and to set up such tests as may serve for the purpose. These methods of exact identification are dealt with in the next chapter, on Bacterial Groups. Similarly examine each set of growths in the other subcultures.

It will often happen that in aerobic hæmoglobin primary cultures there are reddish firm colonies, embedded in the medium, which cannot be subcultured in the ordinary way, as the platinum loop simply slides over them without any part of the growth adhering to it. These colonies, which are perhaps best described as rocky, consist of one or other of three kinds of streptococci, and the first step necessary for the establishment of their identity is to grow them overnight in a serum broth. An isolated colony must be cut out in its entirety from the medium by means of a sharp-pointed platinum wire, and placed in a serum broth tube. At least five or six such serum broth cultures should be made. After incubation of these cultures overnight (sometimes forty-eight hours are necessary), a little of the growth from each must be placed on a slide (marked along one edge as already directed for surface cultures), fixed and stained with Löffler's methylene blue. The criteria of differentiation are given under streptococci in Chapter VI. The method of dealing with suspected colonies of the bacillus of influenza is described in the same chapter, under the influenza bacillus group. There are also one or two other less important instances in which special procedure is required, and these are also dealt with in their appropriate places.

Making and Examination of Anaerobic Cultures.—As a rule, it is essential to study the anaerobic as well as the aerobic flora. Anaerobic methods may be partial, or complete. A partial method, in which oxygen is absorbed by pyrogallic acid and caustic soda, suffices for most purposes. The application of this method to hæmoglobin agar cultures is very simple, occupying less than one minute. The

materials required are a bottle of pryogallic acid, powder or crystals, a solution of 10% caustic soda in water (in a bottle with a rubber stopper through which passes a long glass pipette with a rubber teat at the upper end), a porcelain dish containing hard paraffin, a few corks of a size to fit a test tube of about $\frac{5}{8}$-in. diameter, a pair of scissors, and a pair of broad-pointed dissecting forceps. Place the corks in the vessel containing the paraffin. Heat the paraffin a little above the melting-point by means of a Bunsen flame placed below the porcelain dish. Take the inoculated culture tube in the left hand; cut across the cotton-wool stopper with scissors just above the level of the mouth of the tube. Push the remains of the stopper about half-an-inch down. Pour into the tube some pyrogallic acid powder until it fills about one-third of the space between the top of the cotton-wool and the mouth of the tube. Wet the pyrogallic acid with three or four drops (not more) of 10% solution of caustic soda. Take up one of the corks lying in the melted paraffin with forceps and place it in the mouth of the test tube; push it down until it is quite firm, and hold it in position until the paraffin hardens around it. The culture is now ready for incubation.

A more complete anaerobic condition can be secured by the vacuum method. This requires a Geryk pump, and one or more large, strong wide-mouthed bottles, each fitted with a rubber stopper, through which passes a glass tube with a tap on the other end. The stopper, as well as the junction of the glass tube with the rubber, must be made air-tight by means of resinous ointment, smeared over them before they are placed in position. Some cotton-wool should be laid at the bottom of the bottle. The chamber should be large enough to hold six tubes. After the tubes have been carefully laid within the bottle and the stopper pressed firmly down, the rubber exhaust tube of the Geryk pump is attached to the glass tube of the bottle with the tap open; the air in the bottle is then exhausted by the action of the pump. When this has been done, the glass tap is closed, the rubber tube disconnected and the bottle placed in the incubator. This method is also applicable to broth cultures, but with these the exhaustion must be carried out very slowly.

In some cases it has been found useful to combine the pyrogallic acid and soda method with the exhaustion method.

Another anaerobic method, specially valuable for some purposes, is that of Carrol. It allows a comparatively large quantity of sodium pyrogallate to act upon the air contained in the culture tube, and hence secures stricter anaerobic conditions. The pyrogallic acid and sodium hydrate are placed together in a separate tube of dimensions similar to those of the culture tube, and the two are connected by means of a glass U-tube, united to each by a short piece of rubber tubing of suitable size, previously smeared on the inner surface with vaseline.

Anaerobic cultures are sometimes ready for study after twenty-four hours' incubation, but, as a rule, forty-eight hours are necessary. In order to open a tube put up by the pyrogallic acid-soda method, heat the upper end of the tube over a Bunsen flame until the paraffin begins to melt. Withdraw the cork and lay it down on its broad end in some convenient place. Next, heat the points of the dissecting forceps, and with them catch the cotton-wool plug within the mouth of the culture tube, and pull it out along with the wet pyrogallic acid. A suitable receptacle, containing some antiseptic, should be kept on the table ready to receive these discarded plugs. The operation of drawing out the cotton-wool plugs should be so performed that the whole of the pyrogallic acid, only a portion of which has generally been acted upon by the caustic soda, is swept out of the tube. If a grain or two should still adhere to the rim of the tube, as occasionally happens, the mouth of the tube should immediately be put in the Bunsen flame. The heat will melt and dry up the pyrogallic acid. It is of the utmost importance that no pyrogallic acid should fall into the lower part of the tube.

The use of anaerobic methods in the kind of bacteriological analysis required as a basis for therapeutic immunization is not a refinement, but an absolute necessity. It is impossible to make a complete and satisfactory analysis without obtaining the special evidence yielded by such methods. Experience of cases has shown that facts of essential importance for the understanding of infective conditions will

be missed very frequently, if anaerobic cultures are not systematically made along with aerobic cultures. No small part of the discredit that has fallen upon therapeutic immunization in recent years is due to the incomplete bacteriological analysis that results from the use of aerobic methods alone in the investigation of cases. Many pathogenic bacteria generally regarded as essentially aerobes tend to assume anaerobic habits of growth. They may be present only as anaerobes, refusing to grow, even on blood media, when oxygen is present. Most of the pathogenic aerobic bacteria are liable to occur in this form, but more especially *micrococcus catarrhalis, streptococcus pyogenes, pneumococci* gonococci and diphtheroid bacilli. The pyrogallic acid and soda methods are generally sufficient, but some bacteria require the more rigid anaerobic conditions obtained by exhausting the air in a special chamber by means of the vacuum pump.

In addition to the exact degree of acidity of the medium and the provision of anaerobic conditions, there is one other special requirement for growth that frequently needs to be fulfilled—namely, that of symbiosis. This applies chiefly to the cultivation of the bacillus of influenza, a subject dealt with in the next chapter. Other examples are, however, from time to time observable. Thus there are certainly types of intestinal diphtheroid bacilli that will only grow under the influence of the *bacillus coli communis*. Perhaps the most remarkable thing about these cases of symbiotic growth is that it is not necessary that the micro-organisms should be actually in contact. It is sufficient to place them in proximity, as in adjacent drills.

Staining Methods for Films of Bacteria and Spirochætes

It is very undesirable to multiply staining methods. It is far better to limit oneself to a few and to build up an experience of the microscopical characters of various forms, as they appear when stained in one or two ways. The methods may be reduced to six. The choice of these is not arbitrary. They are the six that experience has proved to be necessary

as aids to bacteriological analysis of the kind required for the purposes of therapeutic immunization.

The best fixative is probably 5% formalin in absolute alcohol, and I recommend that this should be exclusively used.

1. **Gram-Neutral Red.**—This method is used for the differentiation of Gram-fast from Gram-negative bacteria. The staining solutions are (1) one part of saturated alcoholic solution of gentian violet in nine parts of 5% carbolic acid in water, mixed in a test tube shortly before the stain is required; (2) saturated solution of neutral red in water, saturated with thymol. There are also required (3) a solution containing iodine, one part, potassium iodide, two parts, and water 300, and (4) absolute alcohol. The staining process should be carried out as follows:—Fix the dried bacterial film in 5% formalin in alcohol for one to two minutes; wash in water; drop upon the film, through a filter paper in a glass funnel, sufficient of the gentian violet solution to cover it completely, and allow the stain to act for two to five minutes; wash the film well in water; pour over it some of the iodine solution and allow this to act for one minute; wash the film in water; treat with absolute alcohol, until the violet stain ceases to be discharged, or for about one minute; wash the film in water; cover with solution of neutral red and allow this to act for one minute, or longer. Wash thoroughly in water, shake off the moisture and dry slowly over a Bunsen flame. Do not put on a cover glass, unless a permanent preparation is desired.

2. **Alkaline Methylene Blue** (Löffler's).—This stain will be found most serviceable for films of broth cultures of streptococci when these are being examined for length of chain. The stain consists of 30 c.c. of saturated solution of methylene blue in alcohol, added to 100 c.c. of 1 in 10,000 solution of potassium hydrate in distilled water. Fix the film in 5% formalin in alcohol and stain for five minutes in the cold, or for half-a-minute with the slide held over the Bunsen flame, till steam begins to be given off. Wash in water and dry slowly over the Bunsen flame.

3. **Acid Methylene Blue** (Neisser's).—This stain is required as a test for the presence of metachromatic granules. It consists of 2·5 grams of methylene blue dissolved in 5 c.c.

of absolute alcohol, added to 250 c.c. of 5% glacial acetic acid in distilled water. Fix the film in 5% formalin in alcohol, wash in water, and stain for five minutes or longer. The subsequent washing and drying should be carried out rapidly. It is a good plan to rinse for only a second in water, and immediately to blot the film firmly with a folded filter paper, and then to complete the drying at once over the Bunsen flame.

4. **Basic Fuchsine and Methylene Blue for Tubercle Bacilli** (Ziehl-Neelsen method).—The first stain is composed of basic fuchsine, one part; absolute alcohol, ten parts; 5% carbolic acid in water, one hundred parts. The method may be applied either to a simple film or to one prepared from the centrifuge deposit after treatment of the material with antiformin. Antiformin will dissolve nearly everything in a tubercular sputum, except the tubercle bacilli. Its action therefore enables us to obtain a concentration of these bacilli. It consists of equal parts of *liquor sodæ chlorinatæ* and 15% solution of caustic soda. About equal proportions of this and of the sputum should be mixed and thoroughly shaken together in a centrifuge tube. After five minutes, place the tube in the centrifuge for ten to fifteen minutes. Decant the fluid, leaving the deposit at the bottom of the tube. Stir up the deposit with a platinum loop, and add sufficient distilled water and three or four drops of 20% hydrochloric acid in water. Centrifuge again for five minutes, decant the fluid, stir up the deposit as before, and add a second quantity of distilled water. After five minutes' further action of the centrifuge, the deposit is ready for staining. A thin film, either of this deposit or of the original material, should be treated as follows:—Fix for two minutes in formalin and alcohol; wash in water; pour on filtered fuchsine stain; heat over the Bunsen flame until steam arises. Allow the hot stain to act for two or three minutes; wash thoroughly in water; pour on 20% solution of hydrochloric acid in water and allow it to act for about fifteen seconds. Wash thoroughly in water. If the film is still of a deep pink colour, repeat the action of the hydrochloric acid; if, on the other hand, the film is already of a pale pink tint, proceed to counter-stain with Löffler's

methylene blue. Allow this to act for one to two minutes. Wash thoroughly and dry.

5. **Richard Muir's Method of Staining Capsules.**—This method reveals the capsules of pneumococci. Unfortunately, the presence of the capsules is not absolutely diagnostic of the pneumococcus. Some varieties of *streptococcus mucosus* have a similar envelope. The procedure is as follows :—

(1) The film containing the bacteria must be very thin. It is dried and stained in filtered carbol-fuchsin for half-a-minute, the preparation being gently heated.

(2) Wash slightly with spirit and then well in water.

(3) Place in following mordant for a few seconds :—

Saturated solution of corrosive sublimate	2 parts
Tannic acid solution, 20%	2 parts.
Saturated solution of potash alum	5 parts.

(4) Wash well in water.

(5) Treat with methylated spirit for about a minute. (The preparation has a pale reddish appearance.)

(6) Wash well in water.

(7) Counterstain with watery solution of ordinary methylene blue for half-a-minute.

(8) Dehydrate in alcohol, clear in xylol and mount in balsam. (The bacteria are a deep crimson and the capsules of a blue tint.)

6. **Fontana's Silver Method.**—This is probably the best method for showing the presence of spirochætes. It should be carried out as follows :—

(1) Dilute the material to be examined with a drop of water and allow to dry in air.

(2) Pour on, and frequently renew during one minute, a solution composed of acetic acid 1 c.c., formalin 2 c.c., distilled water 100 c.c.

(3) Wash in current of water for several seconds.

(4) Mordant in the following solution :—

Tannin	5 grams
Carbolic acid	1 gram.
Distilled water	100 c.c.

Warm to emission of vapours for about twenty seconds.

(5) Wash the preparation in a current of water for thirty seconds.

(6) Pour on ¼% silver nitrate in distilled water to which ammonia has been added until the precipitate has just dissolved. Warm over Bunsen flame for twenty or thirty seconds, or until the film assumes a deep brown colour.

(7) Wash the preparation in water ; blot with filter paper ; dry and mount in balsam.

CHAPTER VI

BACTERIAL GROUPS AND THE DIFFERENTIAL DIAGNOSIS OF SPECIES

It is intended in this chapter to present in a systematic way the main facts regarding the differential characters of the bacteria it is important to be able to identify in making investigations that have as their object the application of therapeutic immunization to a particular case. Many important pathogenic bacteria have been excluded from the list, for the simple reason that therapeutic immunization has little or no application to the treatment of the diseases of which they are the cause. Numerous details that commonly find a place in systematic text-books of bacteriology are purposely omitted, as not being required.

It is necessary for the accurate analysis of a bacterial flora to have before one a definite scheme of the classification of the important bacteria in groups of closely allied species and varieties. One must know, first, the special characters of each group; and, second, the criteria of identification of the various members of each group.

It is to be frankly admitted that such classifications are based upon incomplete knowledge and therefore merely provisional. They are certain to be gradually modified as knowledge advances. In the meantime, they serve a useful and essential purpose. It is only when we know the group to which a particular bacterium belongs that we can proceed to identify it by the application of distinctive tests, upon which the results of research and experience have led us to depend.

As a rule, it is easy to refer a bacterium to its group. Much additional labour is often necessary before we can definitely identify it as a particular species in this group. Only important and distinctive characters are here given. The bacteriology books are loaded with descriptions of

appearances presented by particular bacteria on various different media, which, though of scientific interest, do not help us to identify the organism, because they are not distinctive.

In the succeeding pages, "on nutrient agar" and "on hæmoglobin agar" always imply incubation at 37° C. for twenty-four hours upon these media, unless otherwise stated. The term "hæmolytic" is used throughout in the sense of *showing power to decompose hæmoglobin in a hæmoglobin agar medium*, and not in the usual sense of *showing power to liberate hæmoglobin from the fresh red corpuscles contained in a blood agar medium*. In my opinion, the latter phenomenon is of comparatively little diagnostic importance, and is apt to mislead. It is impracticable to seek the guidance of both reactions in systematic case-work, and hæmoglobin agar presents so many advantages that I can hardly imagine anyone who has had experience of it willingly abandoning its use.

The groups that it is at present necessary to distinguish are those that may be designated provisionally as follows:—

1. Staphylococci.
2. Streptococci.
3. Coli-Typhoid Bacilli.
4. *Bacillus proteus.*
5. Gram-negative Diplococci.
6. The Bacillus of Diphtheria.
7. Diphtheroid Bacilli.
8. Influenza Bacillus.
9. Tubercle Bacillus.
10. *Diplococcus crassus.*
11. *Bacillus septus.*
12. *Micrococcus tetragenus.*
13. *Bacillus pyocyaneus.*
14. Streptothrices.
15. *Bacillus lepræ.*
16. *Bacillus malleï.*

1. Staphylococci

General Characters.—Staphylococci grow well under aerobic conditions on solid media at a temperature of 37° C., appearing as conspicuous, opaque, soft, rounded, smooth, white or yellowish colonies. They will generally grow under anaerobic conditions, but the colonies are very much smaller. They are Gram-fast, spherical bacteria, averaging about 1 μ in diameter, but sometimes attaining to twice this breadth. They commonly appear aggregated in small clusters. They produce acid in a lactose broth.

Differential Diagnosis from other Groups.—Staphylococci are liable to be confused only with the other Gram-positive cocci. They are *distinguished* from streptococci by their cluster arrangement and the absence of chains in a serum broth culture, from the *diplococcus crassus* group by their much smaller size, and from the *micrococcus tetragenus* group by their arrangement in clusters, instead of in fours.

Classification.—At least three types are to be distinguished :

	Colour of growth on nutrient agar	Mannite	Gelatine at 20° C.
(1) *Staphylococcus pyogenes aureus*	Yellow	+	Liq.
(2) *Staphylococcus pyogenes albus*	White	+	Liq.
(3) Mannite non-fermenters (*a*)	White	−	Liq.
(*b*)	White	−	−
(*c*)	Yellow	−	−

Staphylococcus pyogenes aureus.—It has the special characters indicated in the foregoing table. It is generally rather smaller than any of the other members of the group. Acid is commonly produced in the mannite test broth within twenty-four hours, but occasionally a positive reaction may not be shown until the third day. The appearances of the growth upon nutrient agar, the microscopical characters of the group, and a positive mannite test, suffice for identification. The growth upon hæmoglobin agar is more vigorous than that upon simple nutrient agar, but is not distinct from that of *albus*. It is generally hæmolytic, but not constantly so, and therefore this feature does not serve to distinguish it from other species. It will generally grow under anaerobic conditions, but with diminished vigour. It

is a very important pathogenic bacterium. *Staphylococcus pyogenes citreus* is merely a variety of this staphylococcus.

Staphylococcus pyogenes albus.—This staphylococcus is not essentially different from *aureus* in any particular, excepting that of colour of growth on nutrient agar. Like the preceding, it is a very important pathogenic bacterium. M. H. Gordon (9) has described a white staphylococcus that ferments mannite, but does not liquefy gelatine, and does not form acid in a lactose test broth ; its occurrence is certainly very rare.

Mannite non-fermenting staphylococci.—(*a*) A white staphylococcus giving a negative reaction in mannite, but liquefying gelatine. It is the commonest variety in this sub-group. It is to be identified with *staphylococcus epidermidis albus*, but this is an unfortunate name, as the staphylococcus occurs as a pathogenic agent, not only in the skin, but in the respiratory and urinary tracts. It may be non-hæmolytic, or intensely hæmolytic. It has occasionally been found to grow only anaerobically. It occurs as a saprophyte, but also very commonly as a pathogenic agent of importance.

(*b*) A white staphylococcus giving a negative reaction with mannite and not liquefying gelatine. This staphylococcus is also common and occurs, like the preceding, either as a saprophyte, or as a pathogenic bacterium.

(*c*) A yellow staphylococcus giving a negative reaction with mannite and not liquefying gelatine. This staphylococcus is pathogenic in the skin, but is not of very common occurrence. It has also been found in the nasal passages.

2. Streptococci

The group of the Streptococci is the largest and most important with which we have to deal. It contains numerous pathogenic species, and also several saprophytes.

There is still great diversity of opinion regarding the number of species, their proper nomenclature and the characters by which they are to be distinguished from each other. The fact is that the ascertained distinctive characters of supposed species have hitherto been insufficient for the

purposes of anything approaching a satisfactory classification. The results of the application of biochemical tests have certainly been very important, but the data they supply are not sufficiently numerous or distinctive. Nevertheless, it must be recognized that the work of Gordon (9) and of Andrewes and Horder (19), in 1906, greatly advanced our knowledge of the subject. New lines of division between groups of species have now been provided by the characters presented by various streptococci when grown upon hæmoglobin agar. It is only when these characters are taken together with two or three of the biochemical data, and with the highly distinctive feature of length of chain, that the outlines of a complete and satisfactory classification begin to appear. This classification, which is given below, is one that serves at least as a trustworthy guide in the kind of bacteriological analysis required for the purposes of therapeutic immunization. It has stood the test of several years of continuous practical application without ever having led very far astray. Finality has certainly by no means been reached; further distinctions are certain to be found necessary, as knowledge advances. For example, it is already clear that *streptococcus pyogenes* represents only a large sub-group, in which are included several forms, each distinct in its pathogenic action.

General Characters.—Streptococci are rounded, or slightly elongated, Gram-fast bacteria, which form chains when grown in fluid media. Two elements are generally more closely approximated, so that the chain may be accurately likened to a rosary of pairs of beads. There is a considerable range in size, the smallest being about 0·5 μ in diameter and the largest more than 2 μ. Rod or bacillary forms are quite common in hæmoglobin agar cultures, especially in *streptococcus fæcalis*, *streptococcus pyogenes* and the pneumococcus sub-group.

Differential Diagnosis from other Groups.—Streptococci may be distinguished from other Gram-fast micrococci by their arrangement in short chains of pairs of cocci in surface growths, and their formation of more definite chains in broth cultures. When they exhibit rod forms they may be distinguished from diphtheroid bacilli, with which they are most

liable to be confused, by the absence of metachromatic granules in films stained with acid methylene blue. There is little risk of confusing them with other Gram-positive bacilli, or with Gram-negative bacteria, if correctly stained Gram-neutral red preparations are carefully studied.

Identification of Species.—The most important criteria for the differentiation of species are :—

(1) The characters of the colonies upon hæmoglobin agar under aerobic conditions (rocky or soft ; hæmolytic or non-hæmolytic ; transparent, white, grey, yellow, brown, red or black).
(2) The length of chains in a 24-48 hours' serum broth culture (short or long), and
(3) Positive or negative reaction in lactose, salicin, mannite, inulin, and raffinose test broths.

Hæmolytic action serves only to a limited extent as a criterion for differentiation. It has been clearly established that microscopical characters, with the exception of length of chain, cannot be depended upon as distinctive features. It is perhaps necessary to emphasize the importance of length of chain as a diagnostic feature, and at the same time to insist that the test can be applied satisfactorily only with broths to which serum has been added.

TABLE—

THE STREPTOCOCCI. SEPARATE SPECIES AND THEIR DISTINCTIVE CHARACTERS

Colonies on + 18 *hæmoglobin agar* (*aerobic*)		Serum broth	Chains	Lactose	Salicin	Mannite	Inulin	Raffinose
Streptococcus pyogenes	Usually small, grey, yellow, brown, or transparent, soft and easily broken up; occasionally somewhat firm and detachable as a lozenge. Hæmolytic or non-hæmolytic	Growth usually in a coherent mass or in little particles	Long	+	+ or −	+ or −	−	−
Streptococcus anginosus	Reddish, smooth, rocky, embedded in medium. Usually hæmolytic	Growth in a coherent mass	Long	+	+ or −	+ or −	−	−
Streptococcus fæcalis	Small or large, grey, soft, non-hæmolytic	Copious deposit and cloud	Short	+	+	+	−	−
Streptococcus fæcalis hæmolyticus	Grey, yellow, reddish, brown, or even black; soft; hæmolytic base, or general hæmolysis of medium	Do.	Short	+	+	−	−	−
Pneumococcus— I. II. III. IV. Distinguished only by serum tests	Small, soft, transparent, grey, yellow, brown, or red. Always hæmolytic. Hæmolysis affects the base, and may also extend beyond the edge	Copious deposit and cloud	Short	+	−	−	+ or −	+ or −
Pneumococcus of Rheumatoid Arthritis	Generally larger than the preceding. Otherwise similar. May be intensely hæmolytic or only slightly so	Do.	Short	+	−	−	+ or −	+ or −
Pneumococcus of Pernicious Anæmia	Small, brown, or reddish, intensely hæmolytic. Hæmolysis generally extends through whole of medium	Do.	Short	+ or −	−	−	+ or −	+ or −
Streptococcus salivarius	Small, reddish, smooth, rocky; embedded in medium	Do.	Short	+	−	−	−	−
Streptococcus salivarius, inulin fermenter	Do.	Do.	Short	+		−	+	−
Streptococcus mucosus	Small, colourless, watery, non-hæmolytic	Scanty growth	Short	−	−	−	−	−
Streptococcus equinus	Vigorous, grey, or transparent, non-hæmolytic	Deposit and cloud	Short	−	+	−	−	−

It will be observed that long chains occur only in *streptococcus pyogenes* and *streptococcus anginosus*, that rocky colonies indicate either *streptococcus anginosus* or *streptococcus salivarius* (in one or other of its two forms), and that only *streptococcus fæcalis hæmolyticus* and pneumococci are constantly hæmolytic.

When, in the course of the study of subcultures, it has been established, by observation of the appearances upon hæmoglobin agar and of the microscopical characters, that certain growths are those of streptococci (only occasionally will it be necessary to wait for the evidence of formation of chains before placing them in their group), the procedure necessary for identification of the species is as follows :— Having in your records given each growth its name and number (*strep.* 1, 2, etc.), inoculate a corresponding series of serum broth tubes, transferring an entire colony, if it happens to be rocky. Incubate these tubes for twenty-four hours, or for forty-eight hours, if growth is slow. Stain films of the deposit with alkaline methylene blue and examine for length of chains. The distinction between a short and a long chained streptococcus is usually quite definite. If the chains are long, the diagnosis is established without the application of any further tests, for, if the colonies are rocky, the bacterium can only be *streptococcus anginosus* ; if they are soft, it is *streptococcus pyogenes*. If the chains are short, the evidence of the biochemical tests must be obtained. If the colonies are rocky, the diagnosis now lies between *streptococcus salivarius* and its inulin fermenting pathogenic variety. It will be necessary to set up only an inulin test. If the colonies are soft, all of the five test broths may be inoculated. It has, however, been found that the salicin test generally suffices. If this test is positive, the streptococcus belongs to the *fæcalis* group. If the growth is hæmolytic, it is *streptococcus fæcalis hæmolyticus*. If it is non-hæmolytic, it is *streptococcus fæcalis*. Only very occasionally will doubt arise, in the latter case, as to whether the growth is that of *streptococcus equinus*, for this is very rarely seen in the human subject. A lactose test will settle the question should it arise. If the salicin reaction is negative, and the growth is a hæmolytic one, the bacterium is a

pneumococcus. If the growth is transparent and non-hæmolytic, a diagnosis of *streptococcus mucosus* is justified. Only very rarely, a variety of the pneumococcus of rheumatoid arthritis may be encountered that simulates *streptococcus mucosus*. It may then be necessary to apply the lactose test and to endeavour to obtain also the positive evidence of the inulin and raffinose tests. A pneumococcus that is not definitely hæmolytic on a +18 hæmoglobin agar will almost constantly show distinct hæmolysis on a more alkaline medium, such as a +7·5.

Streptococcus pyogenes.—The distinctive features of this streptococcus are soft colonies on hæmoglobin agar under aerobic conditions, and long chains in a serum broth. Whilst preserving these features, individual strains show somewhat wide differences, some of which, there are good grounds for believing, correspond to differences in pathogenic action. *Streptococcus pyogenes* is therefore probably not a single species, but a sub-group containing several species yet to be defined. In the present position of knowledge we are obliged, for the purposes of bacteriological analysis, to regard it provisionally as a single species with many varieties.

On hæmoglobin agar under aerobic conditions, the colonies are generally small, but in some instances they attain to a breadth of about 2 millimetres. They may be either flat or dome-shaped. Their outline is always circular and smooth. They are transparent, grey, yellow or brown, soft, and generally easily rubbed off the surface of the medium. Occasionally, they are firmer, and, when this is the case, they are often detachable from the medium as a coherent lozenge-shaped body. Rarely, they are comparatively dry and slightly adherent to the surface, but still separable from it. The colonies are generally hæmolytic—in some instances intensely so—but they may be non-hæmolytic. There appears to be no definite relation between hæmolytic power and virulence. Non-hæmolytic types may certainly exercise a powerful pathogenic action. When growing under anaerobic conditions, the colonies also show rather wide variation in their characters; they are less commonly hæmolytic than they are under aerobic conditions. In serum broth cultures, *streptococcus pyogenes* generally forms

a coherent greyish deposit. Some strains grow as more opaque, small particles. Under the microscope this streptococcus has the characters of the group, with the special feature that the chains in a broth culture are long. The individual cocci in some strains are extremely small, whilst in others they attain to the maximum size observed in the class.

Streptococcus pyogenes not infrequently occurs as an important infecting agent in a form in which it will grow only under anaerobic conditions; if these are not given, its presence will remain undetected. Aerobic strains will generally grow under anaerobic conditions, but many strains isolated by anaerobic methods refuse to grow aerobically. They will generally grow in air, however, after passage through a serum broth.

Streptococcus pyogenes attacks the human subject probably more frequently than any other bacterium. Some of its pathogenic actions are of the highest importance as causes of common maladies.

Streptococcus anginosus.—This streptococcus is distinguished morphologically from *streptococcus pyogenes* only in having rocky colonies, instead of soft ones, on hæmoglobin agar under aerobic conditions. For the full development of this character it is necessary that the culture medium should be rich in hæmoglobin and from +12 to +18 in its reaction to phenolphthalein. The colonies are generally fairly large, flat, dry, reddish brown, with a grey top, smooth or rough, always hard and embedded in the medium. If an attempt is made to subculture them in the usual way, it is found that the platinum loop simply glides over them without detaching any portion of the growth. Therefore they cannot be transferred to another hæmoglobin agar surface directly. It is necessary to cut an entire colony out of the medium with a platinum needle, or spud, and to place it in a serum broth. After it has grown overnight in this it can be subcultured on a solid medium. A growth in stroke culture upon hæmoglobin agar can be stripped from the surface as a ribbon, leaving a lacerated surface. If a colony is placed upon a microscopical slide it is found to be hard and granular and extremely difficult to break down into a film. The

growth has generally, but not constantly, a strong hæmolytic action upon the surrounding hæmoglobin medium. Some strains are intensely hæmolytic. Occasionally, this action shows itself in a mustard coloration extending from a group of colonies. This appearance is fairly common in cultures that have been incubated for two days. Under anaerobic conditions on hæmoglobin agar, the colonies are always soft ; they are generally semi-transparent, grey or brown ; they may be hæmolytic, or non-hæmolytic. In serum broth a delicate coherent mass is generally formed at the bottom of the tube in twenty-four hours. There may also be general cloudiness of the broth when it is shaken up.

Streptococcus anginosus sometimes occurs as a pure anaerobe incapable of developing in primary growth under aerobic conditions. If there should be any doubt as to whether an anaerobic growth of a long streptococcus is pyogenes or anginosus, the point may generally be determined by putting it for twenty-four hours in a serum broth and then subculturing upon aerobic hæmoglobin agar. Growth will generally be obtained, and the soft or rocky character of the colonies will then decide the question.

Streptococcus anginosus is a very common and highly important pathogenic bacterium. Its distinction from *streptococcus pyogenes* does not rest alone upon the described difference in the character of the colonies, but also upon the fact that immunization against the one does not protect against the other.

Streptococcus fæcalis.—This is the normal streptococcus of the human intestine. The colonies on hæmoglobin agar under aerobic conditions are generally small, but may attain to a diameter of 2 millimetres. They are grey, soft and non-hæmolytic. Growth can take place under anaerobic conditions. In serum broth there is usually a copious deposit and cloud. Under the microscope, it is generally a short, rounded streptococcus, but some strains grown upon hæmoglobin agar show rod forms. *Streptococcus fæcalis* ferments salicin and mannite, as well as lactose. It can be distinguished by these reactions, its non-hæmolytic colonies and its short chains. One of its special characteristics is that it is

more difficult to kill than most other bacteria with carbolic acid.

Although *streptococcus fæcalis* is a normal inhabitant of the intestinal tract, it can assume a pathogenic action.

Streptococcus fæcalis hæmolyticus.—This streptococcus is sufficiently distinct from *streptococcus fæcalis* to warrant its being regarded as a separate species. Its special characters are its hæmolytic action and its negative reaction in the mannite test broth. The colonies on hæmoglobin agar are generally larger than those of most other streptococci. They are yellow, reddish-brown, or even black. In such growths, rod forms are common. Both under aerobic and anaerobic conditions, the colonies are hæmolytic. Very commonly, especially as it occurs in the nasopharynx, this streptococcus can be isolated only in anaerobic culture. In serum broth, a very abundant deposit generally forms in twenty-four hours. The chains are short. It is distinguished from the pneumococcus by its power to ferment salicin.

Streptococcus fæcalis hæmolyticus is an important pathogenic bacterium, occurring as such especially in the intestine and nasopharynx. The micro-organism described as the "enterococcus" is, for the most part, simply this hæmolytic species of *streptococcus fæcalis*.

Pneumococcus.—Pneumococci grow well upon hæmoglobin agar, and can be subcultured and tested like other species of streptococcus. It is quite unnecessary that the material supposed to contain pneumococci should be injected into a mouse and the micro-organism isolated from the animal's blood after death, as is commonly directed. The group characters are those of a streptococcus that grows on hæmoglobin agar as soft colonies with a hæmolytic base, forms short chains in serum broth and gives a positive reaction with the lactose broth (there are some exceptions), but a negative one with salicin. Positive inulin and raffinose tests, which are not constant, are simply confirmatory ones.

On hæmoglobin agar under aerobic conditions, the colonies are generally small, always soft, and grey, brown or reddish in colour. They are always hæmolytic. The hæmolysis affects the base on which the colony rests, and

generally spreads from it. Occasionally a mustard coloration is produced where the colonies are closely aggregated. Under the microscope the pneumococcus appears as a large streptococcus, generally somewhat elongated. The individuals are commonly, but not constantly, flame-shaped, but this feature is of no value for diagnosis, because it is often shown also by *streptococcus fæcalis hæmolyticus* and *streptococcus pyogenes*, the very micro-organisms from which it is important to distinguish it. The pneumococcus grows vigorously in serum broth, forming a copious deposit ; the chains are short. It is one of the most important pathogenic bacteria ; how wide is its sphere of action is as yet little realized.

It has been specially studied in relation to acute pneumonia, and four different and apparently independent species have been distinguished by means of serum reactions. (See Muir and Ritchie (4), p. 237.)

Primary cultures of pneumococci should always be subcultured as soon as possible, as they tend to die out, owing to the acid conditions induced by other bacteria growing near them.

Pneumococcus of Rheumatoid Arthritis.—This type of pneumococcus differs in no essential particulars from the preceding. In subsequent chapters, evidence will be adduced showing that it is a distinct species, special in its pathogenic action and independent in its immunity reactions.

Pneumococcus of Pernicious Anæmia.—This species of pneumococcus, which is found in the stools of patients suffering from pernicious anæmia, is distinguished by the great intensity of its hæmolytic power, its frequent inability to ferment lactose, and its special reactions in the patient from whom it has been obtained.

Streptococcus salivarius.—The distinguishing features of this streptococcus are rocky colonies on hæmoglobin agar under aerobic conditions, short chains in a serum broth culture, and a negative reaction in an inulin test broth. The colonies are generally smaller than those of *streptococcus anginosus*, and they are rarely, if ever, hæmolytic.

Streptococcus salivarius is a saprophyte of the mouth and nasopharynx.

Streptococcus salivarius, inulin fermenter.—This streptococcus differs from the preceding only in fermenting inulin. It occasionally occurs in persons suffering from rheumatism, and cases favourable for observation have shown that it is one of the several bacteria that may cause, or aggravate, this malady.

Streptococcus mucosus.—This streptococcus is distinguished by its small or even minute, transparent, watery, non-hæmolytic colonies on hæmoglobin agar, its short chains, and negative reactions in lactose, salicin and mannite test broths. It is a saprophyte of the mouth and nasopharynx.

Streptococcus equinus.—This streptococcus forms somewhat vigorous, transparent or greyish, non-hæmolytic colonies on hæmoglobin agar under aerobic conditions. Its chains are short. It gives a positive reaction with salicin, but not with lactose and inulin test broths. It is the *streptococcus fæcalis* of the horse and is rarely found in man, in whom, however, it may act as a pathogenic organism.

Micrococcus rheumaticus. — This streptococcus has been omitted from the foregoing table because there is still doubt as to its being a distinct species. Its described characters do not seem to differ in any essential particular from those of *streptococcus fæcalis*. The contention that it is the cause of acute rheumatism has not been clearly established.

3. Coli-Typhoid Bacilli

This is a large and very important group of pathogenic and non-pathogenic bacilli, the natural habitat of which is the intestinal tract of man and the lower animals. The various members are microscopically indistinguishable from each other. They all appear under the microscope as short rods or threads, the breadth of which varies from about 0·4 μ to 2 μ. They are Gram-negative and non-sporing: they grow rapidly on simple artificial media, such as nutrient agar, on which they appear as large colourless colonies. They possess very active fermenting powers, which differ considerably in each species and thus furnish distinctive tests. Most of the members of the group, but not all, are motile, being provided

with numerous minute flagella. *Bacillus proteus* is somewhat arbitrarily excluded from the group, with which it has as close affinities as have some species that are included in it.

The term "coliform bacillus" is by some restricted to the aberrant members of the *bacillus coli communis* sub-group. The term is more conveniently used as one simply indicating a bacillus of the group, the identity of which has yet to be established. Thus we can speak of the streptococcus flora and the coliform flora of the intestine.

The chief distinctive characters of the more important species contained in the group can with great advantage be set out in comparative tabular form. (See p. 80.) Many such tabulations have been made. The following is based on that given by Muir and Ritchie (4). The corresponding characters of the *proteus* group have been added, as, in laboratory work of the kind required for therapeutic immunization, the question repeatedly arises whether a Gram-negative bacillus under investigation belongs to this group, or to the coli-typhoid one.

Cultivation and Differentiation of Species.—Simple media, such as nutrient agar, will suffice for the cultivation of any of the species. The aerobic growth is vigorous. Although all the members are regarded as facultative anaerobes, the anaerobic growth of some is comparatively feeble and motility generally ceases. This fact can sometimes be taken advantage of when it is desired to separate other bacteria from coliform bacilli.

The general method of procedure already described is quite applicable to the study of this group, but, in work upon typhoid, paratyphoid and dysentery bacilli, it has been found of advantage to use special media that permit of the easy identification of colonies of the lactose non-fermenters. These special media are now numerous. Among the most frequently employed are the Drigalski-Conradi medium (upon which typhoid and paratyphoid colonies have a blue colour, while those of *bacillus coli communis* are red), and MacConkey's bile-salt medium.

In the course of bacteriological investigations of the kind required for therapeutic immunization, we only rarely have to deal with typhoid or dysentery bacilli. It is therefore

DISTINCTIVE REACTIONS OF MEMBERS OF COLI-TYPHOID AND PROTEUS GROUPS

	Motility	Glucose	Lactose	Saccharose	Mannite	Dulcite	Adonite	Sorbite	Litmus Milk			Gelatine	Indol	Voges and Proskauer	Hæmolysis (Hæmoglobin agar)
									Days						
									1	3	15				
Bacillus coli communis	+	AG	AG	–	AG	AG	–	AG	AC	AC	AC	–	+	–	+
Bacillus coli communis, aberrant	+ or –	AG	AG	A	AG	AG	–	AG	AC	AC	AC	–	+	–	+
Bacillus of Friedlander		AG	AG or A	AG	AG	AG	AG	AG	AC or A			–	+		–
Bacillus typhosus	+	A	–	–	A	–	–	A	A	A	A or Alk.	–	–		–
Bacillus paratyphosus A.	+	AG	–	–	AG	AG	–	AG	A	A	A	–	–	–	
Bacillus paratyphosus B.	+	AG	–	–	AG	AG	–	AG	A	Alk.	Alk.	–	–		
Bacillus enteritidis	+	AG	–	–	AG	AG	–	AG	A	Alk.	Alk.	–	–		
Bac. dysenteriæ (Shiga)	–	A	–	–	–	–	–	–	A	Alk.	Alk.	–	–	–	
Bac. dysenteriæ (Flexner)	–	A	–	–	A	–	–	–	A	Alk.	Alk.	–	+		
Bacillus Morgan's No. 1	+	AG	–	–	–	–	–	–	o	o	Alk.	–	+ +	–	
Bac. lactis aerogenes	–	AG	AG	AG	AG	–	AG	AG	AC	AC	AC	–	–	+	
Bac. acidi lactici	–	AG	AG	–	AG	–	AG	AG		AC		–	+	–	
Bacillus cloacæ	+	AG	AG	AG	AG	–	AG	AG		AC		+	+	+	
B. fæcalis alcaligenes	+	–	–	–	–				Alk.	Alk.	Alk.	–	–	–	
B. coli anaerogenes	–	A	A		A				A	A	A	–	+		
B. oxytocus perniciosus	–	AG	AG	AG	AG	AG	AG	AG	AC	AC	AC	+	+	+	
B. MacConkey's No. 71	+	AG	AG	AG	AG	AG	—		AC	AC	AC	–	+	–	
Proteus vulgaris	+	AG	–	AG or	–				Alk.			+	+		–
Bacillus proteus Zenkeri	+	AG	–	–	AC				A			–	+		–

unnecessary to begin the investigation in the way that would be best if we were dealing with suspected or recognized cases of these diseases. We do not need to depart from the general routine described in the previous chapter, unless we encounter a lactose non-fermenter that cannot be included in the *proteus*

group, or identified by its negative glucose reaction as *bacillus fæcalis alcaligenes*. Having obtained pure sub-cultures of coliform bacilli, we need in the first instance inoculate only tubes of lactose and saccharose test broths and of peptone water. If lactose gives acid and gas, saccharose is negative, and indol is formed in peptone water, a diagnosis of *bacillus coli communis* is sufficiently established. If saccharose is rapidly fermented, it will be necessary to apply additional tests, as indicated in the table, to determine which saccharose fermenter we have to deal with. If lactose is not fermented, the first possibility to dispose of is that it is the *bacillus proteus*. The results of glucose, gelatine and indol tests will generally furnish a definite answer and at the same time identify, or exclude, *bacillus fæcalis alcaligenes*. If the bacillus does not give the reactions of either of these, it must belong to the typhoid, enteritidis, or dysentery sub-groups, and a wider investigation will be necessary. If the additional tests indicated in the table are insufficient, it will be best to make a systematic examination of the kind described by Gordon in Abel's *Handbook*, page 116, and by Muir and Ritchie in their *Manual of Bacteriology*, page 388.

Bacillus coli communis.—This is a normal inhabitant of the human intestine. The method of identification has just been described. Whilst most strains give the reactions stated in the table, aberrant forms frequently occur and are apt to cause perplexity. The most common aberrant form is one that ferments saccharose to a slight extent, producing only a faint red tint after two days' incubation. All the true saccharose fermenters produce acid and gas within twenty-four hours. Non-motile forms also occur, indistinguishable from *bacillus acidi lactici*, except by the dulcite and adonite tests.

Although *bacillus coli communis* is normally an intestinal saprophyte, it may be a highly important pathogenic bacterium. It is, indeed, a cause of many forms of serious illness.

Bacillus of Friedländer.—This bacillus, which is capsulated. is of common occurrence and is generally easily identified, The first set of tests already recommended will reveal it as a

saccharose and lactose fermenter. It will be necessary to apply the differential tests for the saccharose fermenters—namely, gelatine, indol and the Voges and Proskauer—and to investigate motility. As *bacillus cloacæ* and *bacillus lactis aerogenes* are by no means rare, the application of these tests is very necessary. Difficulty may arise with aberrant forms that ferment lactose slowly, or not at all. The decision lies, however, only between the bacillus of Friedländer and *proteus vulgaris*, which differs markedly from an aberrant Friedländer in being extremely motile, in liquefying gelatine and in not fermenting mannite.

Like *bacillus coli communis*, the bacillus of Friedländer may be a harmless saprophyte, or an important pathogenic agent.

Bacillus typhosus.—The chief distinctive reactions of this very important pathogenic bacterium are given in the table. As already indicated, when it is encountered in the course of bacteriological work of the kind commonly required for the purposes of therapeutic immunization, it will generally be necessary to resort to special methods of investigation.

Bacillus paratyphosus A and B.—The chief distinctive reactions are given in the table, but should the presence of one or other be suspected, special methods of examination will be necessary, as with *bacillus typhosus*. It is generally stated that type A is rare, whilst the type B is of comparatively common occurrence. Dr A. Rutherford informs me that in India he found that type A was the predominating one.

Bacillus enteritidis (Gaertner).—This is really a large sub-group embracing fairly distinct bacilli pathogenic in man and lower animals. *Bacillus paratyphosus*, A and B, properly belong to it, but have been separated from it on account of their special importance in human pathology. The following species are included in this sub-group :—

B. suipestifer, believed by some to be the cause of swine fever.

Danysz bacillus, pathogenic for rats.

B. typhi murium, pathogenic for mice.

B. psitticosis, pathogenic for some birds.

Bacillus dysenteriæ (Shiga).—The dysentery bacilli also form a fairly large sub-group, the described members of which

differ only slightly from each other in their biochemical reactions. The Shiga and Flexner types are most important. The former is apt to be confused with bacilli of the *proteus* group, from which it is, however, distinguishable by its lack of motility and failure to form gas in the glucose test broth, as well as by other criteria indicated in the table. It also differs from *bacillus typhosus* and *bacillus paratyphosus* in respect of several negative biochemical reactions.

Bacillus dysenteriæ (Flexner).—The biochemical reactions of this bacillus are fairly distinctive. Its positive mannite reaction separates it sharply from *proteus vulgaris*, but there is little save its lack of motility and inability to form gas in a glucose broth to distinguish it from Zenker's species.

Bacillus Morgan's No. 1.—This bacillus has been described by the bacteriologist whose name it bears as one of the causes of summer diarrhœa in infants. It is evidently very closely allied to the *proteus* group. It is especially distinguished by the fact that it produces alkalinity in milk without previous acidity.

Bacillus lactis aerogenes.—This bacillus, like the bacillus of Friedländer, to which it is closely allied, is capsulated. It is distinguished from it by giving a Voges and Proskauer reaction, by inability to produce indol and by a negative dulcite reaction. It is occasionally pathogenic in the human subject.

Bacillus acidi lactici (Hüppe).—This bacillus, one of the common causes of the souring of milk, may have to be distinguished from pathogenic bacteria. As will be seen from the table, its biochemical reactions are sufficiently characteristic.

Bacillus cloacæ.—It is important to recognize this saccharose fermenter, as it is occasionally pathogenic in man. As will be seen from the table, it has reactions that sharply distinguish it from *bacillus coli communis* and the bacillus of Friedländer, the two bacteria to which it is most closely allied, in its pathogenic action.

Bacillus fæcalis alcaligenes.—This species is sharply distinguished by its biochemical reactions. It develops as a vigorous, transparent, watery growth. Under the microscope it is a large bacillus, differing in this respect from *bacillus proteus*. As it sometimes occurs in the nasal

passages, it may have to be distinguished from *bacillus catarrhalis*. Its production of alkali in a glucose test broth is an important aid to identification.

Bacillus coli anaerogenes.—This bacterium is to be regarded as a non-motile variety of *bacillus coli communis*, with comparatively feeble fermentative powers. It is met with from time to time in the course of routine bacteriological examinations and is apt to give rise to temporary difficulty in diagnosis.

Bacillus oxytocus perniciosus.—This bacillus has been described by MacConkey as common in the fæces of man and the lower animals. It is distinguishable by its special biochemical reactions.

Bacillus MacConkey's No. 71.—This bacillus, like the preceding, has been found by MacConkey to be common in the intestine of man and lower animals. Its biochemical reactions are sufficiently distinctive.

4. Bacillus Proteus

This is a group of Gram-negative bacilli so closely allied to the coli-typhoid group that it can be separated from it only on grounds of convenience. The proteus bacilli are common agents of decomposition and putrefaction. They occur, however, quite commonly, both as saprophytes and as pathogenic bacteria, in the human subject. It is therefore of importance to be able to recognize them. It is from time to time necessary especially to distinguish them from typhoid, paratyphoid and dysentery bacilli.

Bacilli of the *proteus* group are distinguished, not by any single character, but by a combination of characters. They are actively motile, Gram-negative rods, or threads; they produce acid and clot (and usually gas) in a glucose test broth, give a negative reaction with lactose, produce indol and (with an important exception) liquefy gelatine.

On nutrient agar, they form a transparent, or greyish, film showing considerable vigour of growth and tending to spread. They are non-hæmolytic. Microscopically they are indistinguishable from bacilli of the coli-typhoid group. They are aerobes and facultative anaerobes. Some varieties are

extremely small, being scarcely larger than an influenza bacillus. Many species have been described, but it is sufficient to distinguish two—namely, *proteus vulgaris* and *bacillus proteus Zenkeri* (see table in preceding subsection). The latter, which is fairly common, is distinguishable especially by its inability to liquefy gelatine. It produces acid in mannite, whereas *proteus vulgaris* does not. The mannite test is important, as it distinguishes *bacillus proteus Zenkeri* from the *bacillus dysenteriæ* of Shiga and Morgan's No. 1. There is little to distinguish *b. proteus Zenkeri* from the Flexner type of dysentery bacillus, excepting its motility and formation of gas in a glucose test broth.

An important practical point that must be borne in mind is that, whilst some varieties of *bacillus proteus* will produce indol rapidly, others will do so only after incubation of the peptone water medium for at least three days. In any case in which a lactose non-fermenting coliform bacillus is found, all of the other differential tests should immediately be set up.

5. Gram-Negative Diplococci

This is a large group containing several important pathogenic species. Its special characters are given by the morphology of the micro-organisms and their Gram-negative reaction. As the name indicates, the individuals occur in pairs. The opposite surfaces are generally somewhat flattened. There is a considerable range in size, the cocci varying from about 3 μ to 0·2 μ, or even less in diameter. The various members of the group are sufficiently distinguished from other micrococci by the two features that have been named.

The group contains such important pathogenic bacteria as the meningococcus, which is the cause of acute cerebro-spinal meningitis, the gonococcus, the micrococcus of Malta fever, and numerous varieties of *micrococcus catarrhalis*, which are the most common causes of acute and chronic catarrhs of the respiratory tract.

Biochemical tests are recognized to be of great value for the distinction of species. The following table shows the chief

members of the group and their important differential characters :—

	Glucose	Galactose	Maltose	Saccharose
Micrococcus intracellularis .	+	+	+	–
Gonococcus	+	+	–	–
Micrococcus Melitensis . .	Alk.		–	–
Micrococcus catarrhalis . .	(or +)		–	–
Micrococcus pseudo-catarrhalis.	+	+	+	+

Micrococcus intracellularis (meningococcus).—This species does not grow upon ordinary agar. It is commonly cultivated, from the cerebro-spinal fluid or other material, on blood agar at 37° C. It is an obligatory aerobe. The colonies generally appear within twenty-four hours as almost transparent, soft, sticky, generally smooth, flat discs. The growths readily die out at room temperature, but they may be kept alive for months at 37° C., if means are taken to prevent drying (Ledingham, *Brit. Med. Journ.*, 13th March 1915). It is stated by Vedder (*Journ. of Infectious Diseases*, May, 1918) that the meningococcus will grow on ordinary agar, if 1% of starch is added to the medium. The individual organisms vary in size, but are in general smaller than the gonococcus. The distinctive biochemical reactions are indicated in the foregoing table. Agglutination of the diplococcus by serum (in dilution of 1 in 50) of a patient who has suffered from cerebro-spinal meningitis may serve to confirm the diagnosis.

Gonococcus.—This diplococcus is difficult to grow, and various special media have been recommended for its cultivation. Human blood agar is commonly used. It grows quite well on +7·5 hæmoglobin agar prepared from sheep's blood, appearing in twenty-four to forty-eight hours as delicate, transparent, or greyish, soft, non-hæmolytic colonies. A matter of the utmost importance for success is the obtaining of the culture material in a perfectly fresh state. Subcultures, which should be made with a larger quantity of

growth than is ordinarily used, require to be incubated for forty-eight hours, or longer. The gonococcus is found in acute and subacute infections as an aerobe, but in very chronic and old-standing infections it can be cultivated only under anaerobic conditions. The pyrogallic acid and sodium hydrate method is best.

Microscopically, this diplococcus is more distinctly bean-shaped than the other members of the group. The distinctive fermentation tests are indicated in the foregoing table. Anaerobic strains are generally of too feeble growth to exhibit these reactions, but they have in several instances been obtained.

Micrococcus Melitensis.—This species is more easy to grow than the two preceding, but its development is very slow. It can be isolated from the blood or urine of persons suffering from Malta fever. In agar culture, it appears as minute transparent colonies in four or five days. On further incubation, the growths assume an amber tint, and finally become of an orange colour. The distinctive biochemical reactions are given in the table.

Micrococcus catarrhalis.—This diplococcus includes many varieties, or sub-species. Some will grow on ordinary agar, but others require a blood, or hæmoglobin medium. *Micrococcus catarrhalis* is commonly an aerobe, but anaerobic types frequently occur and are highly important as pathogenic agents. On agar it appears as a greyish, or transparent growth of moderate vigour. On hæmoglobin agar it is more vigorous, but all degrees of strength of growth are represented in the many varieties contained in the species. The growth is never hæmolytic. A curious grey fogging of the medium is to be observed spreading from the margin of a stroke culture of some varieties.

The microscopical characters are those of the group. There are gradations in size, some varieties being the largest in the group and others the smallest. The most minute forms are anaerobic. In these the diplococcal character is generally lost.

A true *micrococcus catarrhalis*, according to the usual description, should give negative reactions with all of the four test broths employed for the differentiation of the species

in the group. Many pathogenic strains produce, however, a small amount of acid from glucose, and it is important not to relegate them to the *pseudo-catarrhalis* group when this is found to be the case.

With considerable frequency, in the course of routine work in the Laboratory of the Scottish Asylums, there has been isolated from the respiratory tract or buccal cavity, a short, oval bacillus that cannot be separated from this species. It has all the characters of *micrococcus catarrhalis*, excepting its bacillary shape, which depends simply upon elongation of the coccus. It is apt to be mistaken for a coliform bacillus. The only member of this group that it really resembles is, however, *bacillus fæcalis alcaligenes*, from which it is distinguished in being non-motile and in not forming alkali.

Micrococcus pseudo-catarrhalis.—This is a large sub-group of Gram-negative diplococci found commonly in the mouth and the respiratory tract. Its members are supposed to be non-pathogenic, but there are good grounds for believing that some types have a pathogenic action similar to that of a true *micrococcus catarrhalis*. The characters of the growth do not differ essentially from those of *micrococcus catarrhalis*. They may be very vigorous, or extremely feeble. Microscopically, there is no distinctive feature. We have to rely upon the biochemical tests. Most types of pseudo-catarrhalis diplococci ferment all of the four sugars used for the differentiation of the species in the group. It is generally sufficient, however, to ascertain that acid and clot are produced in the glucose and saccharose test broths.

6. The Bacillus of Diphtheria

This group contains only a single species, the *bacillus diphtheriæ*, or the Klebs-Löffler bacillus. It is sharply distinguished from numerous other bacilli, morphologically very similar, almost solely by its special pathogenic actions. These morphologically similar species have been designated pseudo-diphtheria bacilli, but many of them are far too important in human pathology to be regarded merely in their negative relation to the bacillus of diphtheria. They are

here taken as a separate group, under the name Diphtheroid Bacilli, now commonly employed.

The bacillus of diphtheria grows best upon a serum, or hæmoglobin, agar medium under aerobic conditions. It is, however, a facultative anaerobe. It forms soft, grey colonies of moderate vigour, which appear in from six to twenty-four hours. In serum broth, it grows well, producing a general turbidity. It is non-motile. It is one of the few pathogenic bacteria that derive their virulence from the production of an exotoxin.

In Gram-preparations, the appearance is that of Gram-fast rods, somewhat irregular in shape and size, and often enlarged, or clubbed, at one end. The average length is about 3 μ. The bacilli may appear incompletely Gram-fast, and in this case have a granular aspect. In acid methylene blue preparations they are seen as pale blue rods, containing one or two deep purple granules, which are generally referred to as metachromatic granules.

The important biochemical reactions are as follows :—

Glucose	*Lactose*	*Saccharose*	*Dextrin*
A.C.	A.C.	–	A.C.

Many bacilli of the diphtheroid group ferment glucose, lactose and saccharose, but it would appear that only the bacillus of diphtheria produces acid from dextrin (Hewlett and Hine). This dextrin reaction certainly seems to be an important, if not absolutely distinctive one. It is said, however, that it is not given by some virulent strains. In order to establish a diagnosis, recourse has generally to be had to subcutaneous inoculation of a guinea-pig, with 1 c.c. of a twenty-four-hour broth culture. If the suspected culture is that of a virulent diphtheria bacillus, the animal will die within about thirty-six hours. Morphology alone should not be depended upon for diagnosis, as the microscopical characters are very closely simulated by several members of the diphtheroid group. A positive dextrin reaction and virulence to the guinea-pig are the only criteria that really serve for identification. Non-virulent strains may be met with, giving a positive dextrin reaction, but failing to kill a guinea-pig.

7. Diphtheroid Bacilli

In this group are included numerous species of bacilli, having a morphological resemblance to the bacillus of diphtheria in respect of containing metachromatic granules, but differing from it in being non-virulent to the guinea-pig, and in failing to produce the same exotoxin. All bacilli showing metachromatic granules, but not conforming to the characters of the *bacillus diphtheriæ*, or of the streptothrices, are to be regarded as members of the group. Gram-fast bacilli which contain no metachromatic granules, such as *bacillus septus*, do not belong to it.

Diphtheroid bacilli are extremely common in the mucous secretions, especially in association with chronic inflammatory disorders of the respiratory, alimentary and genito-urinary tracts. They may be harmless saprophytes, or pathogenic bacteria of great importance. So common are they that it is practically unnecessary even to suspect them of being the bacillus of diphtheria, unless there are clinical symptoms suggestive of diphtheritic poisoning. The ordinary criteria for the differentiation of species are of little value for this group, on account of the remarkable inconsistency of both morphological and biochemical characters, which may vary considerably in different subcultures of apparently the same pure growth, and may even change in successive subcultures. Great and, as yet, insuperable difficulties therefore stand in the way of differentiation of the individual members of the group. Nevertheless, there is ample evidence that many distinct species exist, and that various members of the group are pathogenic agents of high importance in human pathology. This subject of the pathogenic action of diphtheroid bacilli is fully discussed in Chapter X.

The diphtheroid bacilli that may be isolated in the course of ordinary case investigations vary greatly in certain particulars. They may be aerobic, or anaerobic. The growths may be vigorous, or weak; they are generally grey, but may be colourless, or yellow. They are commonly non-hæmolytic, but hæmolytic types are occasionally found and are of special pathogenic importance. There are large and

small types of bacilli, some being as minute as the influenza bacillus. They may occur as separate rods, or be united in chains. The metachromatic granules may be few in number, or they may pack the body of each bacillus. These granules also vary in size, some being so minute as to be recognized only with difficulty, whilst others distend the bacillary body. The bacilli may be strongly Gram-fast, or (though rarely) Gram-negative. Some strains ferment glucose, saccharose, lactose and mannite readily, whilst others seem unable to produce any acid from these substances. There are grounds for believing that, in some instances, the puzzling polymorphism of some strains of diphtheroid bacilli is due really to a symbiosis of two or more different species. This is a matter that requires investigation.

Only two species can be separated out with any definiteness—namely, a catarrhal diphtheroid bacillus, and *bacillus acnes*. The others, by far the larger proportion, await differentiation by criteria still to be discovered. Though we cannot differentiate them from other species, it is possible already to recognize that there are forms that display powerful neurotoxic actions in the human subject, constituting the essential pathogenic agent in a large group of cases of neurasthenia, as well as in exophthalmic goitre, and some forms of acute and chronic insanity.

Catarrhal Diphtheroid Bacillus.—That a particular species of diphtheroid bacillus is one of the several bacterial causes of acute coryza has been clearly established. The evidence will be detailed in Chapter X. This species can be defined to a certain extent, but it cannot be absolutely differentiated from some others. It is a short, fairly large bacillus, strongly Gram-fast. With Neisser's stain, it generally shows one or more metachromatic granules in each element. On simple nutrient agar, it grows as soft, greyish colonies. On hæmoglobin agar it forms fairly large, rather dry, dense, whitish and slightly yellow, non-hæmolytic growths. It is one of the diphtheroids that may show a fogging of the hæmoglobin medium beyond the growth, whilst underneath the normal appearance is retained. As a rule it produces acid and clot in glucose and saccharose test broths.

Bacillus acnes.—This bacillus can be grown from the inner

end of an expressed comedo, or from an acne pustule. It requires an acid medium, from +30 to +40 being best, and anaerobic conditions. It grows well on a +30 hæmoglobin agar, set up by the pyrogallic acid and caustic soda method. The colonies, which develop in about forty-eight hours, are whitish and often somewhat difficult to distinguish in the primary cultures from those of accompanying staphylococci. After its growth has been continued under anaerobic conditions through two or three subcultures, the bacillus will generally grow aerobically on the same medium. Stained by Neisser's method, it is a pale blue rod with one or more deeply stained granules within it. These granules are blue-black, rather than purple. Short threads containing six or more granules are common. Coccal forms also occur. In Gram-neutral red preparations the granules are Gram-fast, and the rest of the bacillus is of a pale violet tint. There are grounds for believing that there are several varieties, differing in growth-characters and in morphology. If oleic acid is added to the medium, a more vigorous growth results, but the bacillus loses its diphtheroid character (J. J. Ritchie). According to Sudmersen and Thompson (*Journal of Pathology and Bacteriology*, October, 1909), the acne bacillus is virulent to mice, but not to guinea-pigs.

There are three named varieties of "pseudo-diphtheria bacilli," frequently mentioned, that require some reference in this place. They are Hofmann's bacillus, the Xerosis bacillus, and the Morax-Axenfeld bacillus.

Hofmann's bacillus, said to be present commonly in the nasal passages, is generally described as a Gram-fast rod with some resemblance to the diphtheria bacillus, but without clubbing, devoid of metachromatic granules, and giving negative reactions with the test broths. If it is really devoid of metachromatic granules it should not be included in the diphtheroid group. In my experience, a culture of the bacillus that corresponds most closely to that described as Hofmann's bacillus can always be shown to contain some individuals with metachromatic granules. Inability to produce acid in the test broths is a feature shared by many strains of diphtheroid bacilli with abundant metachromatic granules. The described characters do not clearly differen-

tiate Hofmann's bacillus from bacilli of the diphtheroid group commonly found in other situations. Whether it is entitled to be regarded as a separate species, or not, can be determined only when satisfactory differential criteria for the members of the group have been discovered and applied.

The Xerosis bacillus is variously described, some authorities saying that it contains metachromatic granules and others that it does not, some stating that it produces acid from glucose and saccharose and others that it ferments none of the sugars. The name has undoubtedly been applied to many different species of diphtheroid bacillus isolated from the various regions in which members of the groups are commonly to be found.

The Morax-Axenfeld bacillus is a diphtheroid bacillus—a fact not always recognized—and it may be doubted it the micro-organism to which various observers have assigned the name has always been of the same species.

8. Influenza Bacillus

This group contains three species, *bacillus influenzæ*, *bacillus pertussis* (of Bordet-Gengou), and the Koch-Weeks bacillus. All are difficult to grow, at least in subculture, and require the fulfilment of special cultural conditions.

The group characters are those of a Gram-negative bacillus, extremely minute, devoid of metachromatic granules, not fermenting glucose, and growing only under aerobic conditions.

The only other bacteria that are liable to be mistaken for bacilli of the influenza group are some minute diphtheroids that are Gram-negative and some varieties of *bacillus proteus*. The metachromatic granules of the former (which, moreover, are almost always anaerobes), and the very free growth of the latter, as well as its formation of acid in glucose broth, serve for correct differentiation.

Within the group, practically the only distinction that has to be made, and it is one that is sometimes extremely important, is between the bacillus of influenza and the Bordet-Gengou bacillus of whooping-cough. The claims that some

authorities make to being able to distinguish them by differences of morphology are probably ill-founded. The only criterion that holds, in my experience, is that, whilst the Bordet-Gengou bacillus will grow in subculture on hæmoglobin agar alone, the bacillus of influenza will not do so, but requires the support of other bacteria.

Bacillus influenzæ.—It has the characters of the group. When growing under difficulties—that is to say, without sufficient support from other living bacteria—it tends to assume thread forms. Some strains show deeper staining at the ends, and thus come to resemble somewhat closely the minute types of *micrococcus catarrhalis*. The whiter and more vigorous growth of the latter upon hæmoglobin agar, and its ability to grow alone, will generally serve to settle the diagnosis. In primary growths on hæmoglobin agar the colonies of *bacillus influenzæ* appear in twenty-four hours as extremely minute, transparent, watery, non-hæmolytic colonies, which disappear on being touched with a platinum wire. They tend to be specially numerous in the immediate vicinity of colonies of other bacteria, especially those of the *catarrhalis* group. Subcultures must be made on hæmoglobin agar in drills, alternating with drills of other bacteria (J. J. Ritchie). It is of the utmost importance that the surface should be free from water of condensation. The best supporting growths are *micrococcus catarrhalis*, pseudo-catarrhalis diplococci, and pneumococci.

Bacillus pertussis.—The general characters are those already described for the bacillus of influenza. There is certainly a greater tendency to the occurrence of diplococcal forms. Whilst this bacillus may be grown in pure subculture upon hæmoglobin agar, its vigour is greatly increased by the support of catarrhalis diplococci, as in the case of *bacillus influenzæ*.

Koch-Weeks Bacillus.—This bacillus occurs only in connection with acute and chronic conjunctivitis and may thus be distinguished by the source from which it is obtained. It is the smallest bacillus in the group, measuring little more than 1 μ in length. Its colonies resemble those of the two other species. It is always accompanied by diphtheroid bacilli, in symbiosis with which it probably grows.

9. Tubercle Bacillus

This group includes three closely allied varieties of *Bacillus tuberculosis*, known respectively as the human, bovine and avian types. The group characters are those of an acid-fast bacillus, non-motile and non-sporing, which will not grow on the ordinary media, which develops very slowly and in characteristic fashion upon media suitable for its growth, and which is pathogenic in animals of the class to which it is specially related, producing characteristic tubercular lesions.

Cultures are best made upon Dorset's egg medium. If the culture material is known to contain other bacteria, these should be destroyed by antiformin before the cultures are made. For example, phthisical sputum should be mixed with about twice its volume of antiformin solution and thoroughly shaken up. After the elapse of a quarter of an hour or so, the mixture is centrifuged for from five to ten minutes. The fluid portion is then decanted and the deposit washed with two changes of normal salt solution, the centrifuge being again used to throw down the deposit. Cultures are made from the washed deposit. The cotton-wool plugs must be dipped in melted hard paraffin, in order to seal the tubes and so prevent evaporation. The growths appear after three or four weeks' incubation at 37° C., as whitish colonies. Incubation should be continued for some weeks longer, during which time the amount of growth gradually increases.

Bacillus tuberculosis, human type.—This bacillus is said to average 3·5 μ in length and 0·3 μ in breadth. The rods are straight or curved, and sometimes slightly enlarged at one end. On Dorset's egg medium, the fully developed growth is dry, tough, wrinkled and white, or yellowish. Inoculation into a guinea-pig causes death of the animal in about six weeks from general tuberculosis. Inoculation of 0·1 mg., or less, of the culture into the vein of a rabbit does not result in the development of tuberculosis, thus contrasting with the action of the bacillus of bovine tubercle.

Bacillus tuberculosis, bovine type.—This bacillus is shorter and thicker than the preceding. Its growth on Dorset's egg medium is less vigorous, appearing as a thin, moist, whitish layer, easily broken up. It is even more virulent to the

guinea-pig than the human type. Injected into the vein of a rabbit, it causes death from general tuberculosis.

Bacillus tuberculosis, avian type.—This type cannot be distinguished morphologically from the other two. It grows more vigorously, but requires a higher temperature (43·5° C.). It is not virulent to the guinea-pig.

There are some other acid-fast bacilli which must not be mistaken for the tubercle bacillus. They are chiefly the timothy grass bacilli, the smegma bacilli, and Johne's bacillus (bacillus of chronic bovine pseudo-tuberculous enteritis). The grass bacilli grow easily and quickly on simple media, but the two other types require rather special conditions for their successful culture. Practically, however, there is little danger of being misled in the study of tubercle bacilli by these other acid-fast bacteria. Microscopically, the smegma bacilli can be distinguished by the fact that they are decolorized by alcohol (after treatment of the film by acid) in the Ziehl-Neelsen staining method. In case of doubt, the cultural and virulence tests will furnish decisive criteria.

10. Diplococcus crassus

This is a group of large, Gram-fast diplococci, various members of which occur as common saprophytes in the mouth, respiratory tract, skin, and lower portion of the genito-urinary tract. Some species are capable of assuming a pathogenic action. The group characters are those of a Gram-fast, bean-shaped diplococcus, larger than a staphylococcus and not forming clusters, but sometimes growing in short chains in broth. The appearances on solid culture media vary considerably. The growths are generally white, but some strains are vigorous, whilst others are very delicate. No differentiation of species appears yet to have been made, nor do the biochemical reactions, by which the group might be distinguished more sharply from staphylococci and streptococci, seem to have been worked out.

11. Bacillus septus (Cautley's Bacillus)

This is a Gram-fast bacillus, occasionally found in the nasal passages. It is one of the several infective causes of coryza. On simple agar, and also upon hæmoglobin agar, it forms large, whitish opaque colonies. It is non-hæmolytic. Under the microscope, in a Gram-neutral red preparation, it appears as a deeply stained short rod with rounded ends. The staining is generally less deep in the centre. Stained by Neisser's method, it is a pale blue rod, devoid of metachromatic granules. It produces acid in glucose, lactose, saccharose and maltose test broths. It is distinguished from bacilli of the diphtheroid group by the absence of metachromatic granules, and from Hofmann's bacillus especially by its positive biochemical reactions. Benham (20), who has given an otherwise admirable description of it, speaks of it as a diphtheroid bacillus. To include bacilli devoid of metachromatic granules in this group can lead only to confusion.

12. Micrococcus tetragenus

This is a small group of Gram-positive diplococci, distinguished by their arrangement in groups of four. They are pyogenic bacteria, occurring chiefly in the mouth and respiratory tract. There are at least two species, *micrococcus tetragenus* and *micrococcus paratetragenus*. They form acid in most of the common test media. They do not liquefy gelatine.

Micrococcus tetragenus.—This species is smaller than the other. On nutrient agar it forms rather small, white, slightly elevated, moist colonies.

Micrococcus paratetragenus.—This species is distinguished from the other chiefly by its larger size. The colonies on nutrient agar are dry, instead of moist, and they tend to adhere to the medium. On hæmoglobin agar they have a bright yellow colour. It decolorizes rather easily in the Gram-staining process, if the action of the alcohol is too long continued. It is thus liable to be mistaken for a Gram-negative diplococcus. Benham (20) states that it produces acid in glucose, saccharose, lactose and maltose test broths.

13. Bacillus pyocyaneus

This is a group of Gram-negative bacilli presenting very distinctive characters, and of considerable pathogenic importance. They are slender rods, actively motile and Gram-negative. They grow readily in ordinary culture media, producing whitish, soft colonies. The growth imparts to the medium a characteristic yellow-green or bluish tint. Gelatine is liquefied and milk coagulated. The pigment is soluble in chloroform; it changes to red on addition of a weak acid. The formation of this pigment serves to distinguish *bacillus pyocyaneus* from bacilli of the coli-typhoid group. It is said to be a facultative anaerobe, but in my experience it has refused to grow anaerobically.

In addition to the type represented by the common pathogenic form of *bacillus pyocyaneus*, the following two other species, probably non-pathogenic, have been described.

Bacillus fluorescens liquefaciens.—It does not clot milk.

Bacillus fluorescens non-liquefaciens.—It does not liquefy gelatine.

14. Streptothrices

The streptothrix group includes numerous aerobic and anaerobic species. The important pathogenic forms are mostly anaerobes. The general characters are those of a filamentous micro-organism, typically composed of Gram-positive, unsegmented, branching threads, but always displaying also segmented, or bacillary and coccal forms. The filamentous forms have metachromatic granules.

There are evidently numerous species of streptothrix yet to be accurately identified.

Streptothrix actinomyces.—The anaerobic form is the ascertained cause of actinomycosis. There are numerous sub-species. The *actinobacillus* is a bacillary form cultivated from cases of actinomycosis; it shows no filaments. Whether it is distinct from the threading form, or merely one of its phases, seems yet undetermined.

M. H. Gordon (*Brit. Med. Journ.*, 27th March 1920) recommends growing this micro-organism in ordinary nutrient broth to which a few drops of fresh human blood have been

added. Some of the cultures should be placed under anaerobic conditions by covering the broth with a layer of oil. After incubation for a few days, small white masses appear at the bottom of the tubes.

Streptothrix maduræ.—This is the cause of madura foot. It seems to be identical with *actinomyces.*

Streptothrices associated with Diabetes Mellitus and Allied Morbid Conditions.—For several years I have had under observation the occurrence of an anaerobic streptothrix in the stools. I described it, in 1919, as a prominent element in the intestinal flora in cases of diabetes mellitus (47). In hæmoglobin agar cultures, put up anaerobically by the pyro-soda method, it appears in from twenty-four to forty-eight hours as small or medium-sized greyish colonies, generally with ragged edges, and having the aspect of powdered sugar that has been wetted. It grows, as a rule, on a +18 medium, but it would sometimes seem to prefer a more alkaline one. Occasionally, a strain is encountered that can be continued in subculture only with difficulty. Under the microscope, it appears as Gram-fast filaments with occasional joints. It often, however, assumes a purely bacillary form, somewhat resembling the bacillus of anthrax. Some streptothrices isolated from cases of diabetes have shown a remarkable ability to split up starch into a substance that will reduce Fehling's solution, but I am not now inclined to attach very much importance to this action as bearing upon the pathology of diabetes mellitus. So far, I have found a streptothrix of this kind in the stools in every case of diabetes mellitus examined. A similar streptothrix has, however, been obtained from cases that were not of this nature, but in which there was, in nearly every instance, found to be glycosuria. The probable pathogenic action of this bacterium is considered in Chapter X.

15. Bacillus lepræ

That this bacillus, which closely resembles the tubercle bacillus, is the cause of leprosy seems now to be satisfactorily established, although many points are still in dispute. For the many details connected with the subject and the method

of cultivation, the reader is referred to the systematic text-books of bacteriology. (See, for example, Muir and Ritchie (4), page 301.)

16. Bacillus mallei

The glanders bacillus is a non-motile Gram-negative rod of about the size of the tubercle bacillus. It grows readily on various media. (See Muir and Ritchie (4), page 309.)

A bacterium about which it is necessary to add a special note is the widely distributed non-pathogenic *bacillus subtilis*. It is the bane of the bacteriology laboratory, resisting sterilization and often turning up in the most unexpected fashion in media supposed to be sterile. It is an aerobic, threading, sporing bacillus, actively motile, and growing freely on all media as a transparent or greyish spreading film, or as whitish colonies. It is easily recognized under the microscope by its large oval, generally unstained, spores. It is abundant in hay and straw, and consequently may readily contaminate glass that has been packed in this material. For the same reason it is abundant about the wool of the sheep, and therefore special precautions have to be taken against contamination of the tubes in which blood is collected from this animal. The spores are extraordinarily resistant. Prolonged boiling may have no effect upon them. They have even been known to come out of the autoclave alive, after having been subjected to a temperature of 120° C. for an hour. Tubes that have been contaminated by *bacillus subtilis* should be collected and placed in the autoclave at 130° C. for one hour, or longer.

CHAPTER VII

THE CLINICAL INVESTIGATION OF INFECTIONS

THE methods generally employed in order to obtain culture material from various supposed seats of infection are those that the observations and experience of many workers have proved to be the best. They vary considerably in accordance with the locality and probable nature of the infections.

As a rule, before cultures are made, the culture material should be studied under the microscope in a film spread on a glass slide and fixed and stained by suitable methods. If the material is thick, it is generally necessary to mix it with a drop of normal salt solution placed on the slide. The film must be dried, either at room temperature or with the aid of very gentle heat, and then fixed in alcohol, or 10% formalin in alcohol. For staining, Gram's method is the most generally useful, but Löffler's methylene blue is better for some kinds of material. In some instances other stains are necessary, as, for example, Neisser's methylene blue for the detection of diphtheroid bacilli, Muir's capsule stain for pneumococci and the Ziehl-Neelsen method for tubercle bacilli. There is not always time to make direct films, and they may often be dispensed with. The chief purpose of the examination of a direct film is to enable the bacteriologist to gain an idea of the kind of bacteria present, and also of their number, so that he may have some guidance in regard to the extent to which the material should be diluted in making the cultures. It must be understood that a direct film alone can never serve for the analysis of a bacterial flora. It is a common experience that important pathogenic species, the presence of which was not suspected from the examination of the direct film, make their appearance in the cultures.

All swabs that cannot be used immediately they are taken must be placed in agar immersion tubes. If they are allowed to dry, they are useless for cultural purposes.

1. Respiratory Tract

In cases of suspected bacterial infection of the upper respiratory tract, we may examine either specimens of the discharged secretions, or swabs taken from the nasal passages, nasopharynx, accessory sinuses, or Eustachian tube. A swab taken from the region of the inferior turbinals is generally more satisfactory for bacteriological examination than a specimen of the nasal discharge, which is apt to be too greatly diluted with mucus and to be contaminated by contact with the external nares. As a rule, a nasal swab should be taken through a speculum. Post-nasal swabs should be taken through the mouth, and the utmost care must be exercised to avoid touching any surface except that from which the specimen is required. Whilst a swab is generally quite satisfactory, experience has shown that it may occasionally prove defective, and that a specimen of post-nasal sputum, which must have come from the infected area, gives more uniformly successful results. Indeed, if sputum can be obtained, it is scarcely worth while taking a swab. To obtain special culture material from the antrum, frontal sinuses, ethmoidal sinus, or the Eustachian tube, is a matter of no small difficulty that had best, as a rule, be left to the rhinologist.

In cases of bacterial infection of the lower respiratory tract, we have to depend wholly, except perhaps in some special cases of laryngeal infection, upon specimens of sputum. The chief source of contamination is the mouth, and, if the expectoration can be produced at will, the patient should, before voiding it, use a peroxide of hydrogen mouthwash, or, if this is not available, simply rinse out the mouth and gargle the throat with warm water. A sterilized specimen tube is the most convenient receptacle for sputum.

Swabs procured at a distance from the Laboratory should always be placed in an agar immersion tube to prevent drying. Whilst it is desirable that all specimens from which cultures are to be made should be as fresh as possible, quite satisfactory results may generally be obtained from them if they are not much over twenty-four hours old, provided measures have been taken to prevent them drying up, and

that they are kept in the cold. It is specially important that all culture material from the respiratory tract should be examined in direct film before cultures are made. The extent to which the material is to be diluted, in order to obtain cultures with discrete colonies in sufficient numbers, will depend chiefly upon the profusion or paucity of bacteria visible in such a film. In all cases of chronic bronchial infection the sputum should be examined for tubercle bacilli.

A nasal swab is generally comparatively dilute as regards its bacterial content, and cultures may be made by simply rubbing it over the agar and hæmoglobin agar surfaces. Great care must be taken, however, not to scratch the medium, as, if this is done, some types of delicate colony may be difficult to recognize. In cases of acute coryza, only aerobic cultures need be made, as anaerobic bacteria are never in my experience a cause of this malady. In cases of chronic nasal catarrh, and especially when infections of the accessory sinuses or Eustachian tube are being investigated, it is of the utmost importance that anaerobic, as well as aerobic, methods be used. The culture material provided by means of a post-nasal swab must always be regarded as requiring great dilution. If it is put directly upon the medium only confluent growths will result. The best plan is to use an agar surface as a place for spreading and mixing the material, and then to transfer a small portion of it to hæmoglobin agar tubes. In order to make sure of the development of discrete colonies, the platinum loop, after having served to inoculate one hæmoglobin surface, may be carried on to another.

Post-nasal and bronchial sputum must be washed in several changes of sterile normal salt solution with the aid of gentle or vigorous shaking, according to the delicate or tough consistence of the material. When the sputum has ceased to leave any opacity in the fluid, as much as will fill a 1 m.m. platinum loop, taken from one of the denser portions, should be transferred to an agar surface. On this it must be thoroughly broken up, mixed and spread out as a thin layer. Hæmoglobin agar surfaces should then be inoculated sparsely or freely, in accordance with the opinion formed as to the

number of micro-organisms present from the examination of the direct film. The fractional portion of culture material carried over to the hæmoglobin agar must be thoroughly spread over the surface, and, if economy of medium is not an important consideration, it is well to carry the loop over to a second surface. The number of aerobic and anaerobic cultures set up must depend upon the nature of the case. As a rule, a slightly alkaline medium (+18) suffices for all purposes, but the detection of a pneumococcus infection is facilitated by the inoculation of a +6 hæmoglobin agar medium. Acid media are hardly ever required for cultures from the respiratory tract. All cultures should be placed in an incubator at 37° C., immediately after they have been set up. The further procedure has already been described in Chapter V.

2. Alimentary Tract

Bacteriological investigation is almost limited to two kinds of material—namely, that obtainable through the mouth, and the intestinal evacuations. I believe that the systematic bacteriological examination of stomach washes would give important information in cases of gastric disorder, but, regarding this matter, I cannot speak from experience. It has, however, been proved in numerous cases that the flora of the stools may give valuable indications of the existence of a gastric or duodenal chronic infection, which may be confirmed by focal reactions after injection of doses of an autogenous vaccine. The surgeon has many opportunities of gaining information regarding the flora of commonly inaccessible parts of the alimentary tract in morbid and normal conditions, but it is not yet his custom to make systematic use of them. His neglect of the infective element in many of the morbid conditions that he is ever ready to rush at with knife and needle is to-day laying him open to adverse criticism, for it is certainly his duty to utilize all available knowledge that may be of advantage to his patient. For example, very many cases of gastric and duodenal ulcer are operated upon that are amenable to treatment by therapeutic immunization ; and chronic intestinal disorders that are equally controllable by similar means are allowed to become aggravated

without a thought of their bacterial causes, until, as a last resort, the patient is subjected to a serious operation that rarely removes completely the real cause of the trouble. Many post-operative cases of this kind have passed through my hands, and received that benefit from therapeutic immunization that they might have experienced before and without operation.

The Mouth.—Cultures are frequently required from the gums, or, more exactly, from alveolar spaces, in cases of pyorrhœa alveolaris. The bacteriological examination is much facilitated by careful cleansing of the gums before a specimen of the pus is taken. The use of a mouthwash, such as a solution of peroxide of hydrogen, is often sufficient, but one of the best plans is to wipe the gums with a pledget of cotton-wool soaked in absolute alcohol. The pus may thereafter be squeezed from the alveolar pocket by pressure on the gums with the fingers, or a specially flattened swab may be inserted between the tooth and the gum. If the culture tubes are at hand, a flattened platinum wire may be used instead of a swab. As swab specimens dry very quickly, they should always (except when the cultures can be made at once) be plunged into an agar immersion tube. Pyorrhœic pus is always loaded with bacteria, and extreme dilution of it is necessary in order to obtain discrete colonies. This dilution is best made on an agar surface in the way already described in the preceding subsection. Anaerobic cultures should never be omitted.

Cultures may also be required from the tonsils, from inflamed patches about the buccal mucosa, from the tongue and from the pharynx. If possible, a peroxide of hydrogen mouthwash and gargle should be used before a swab is taken. We do not wish a sample of the mucus lying on the surface, but a scraping of the epithelium. Therefore, after the mucus has been washed off, the swab should be taken with the application of some force. Extreme dilution of the material is almost always necessary.

The Gastro-Intestinal Tract.—We have to depend upon the analysis of the bacterial flora of the stools. Only on rare occasions, provided by the surgeon, can cultures be made from the mucous surface of any part of the tract. The

systematic study of the flora of the stools is, however, capable of yielding information of great practical importance, and may even be made to enlighten us regarding the probable infective cause of a lesion so distant as a duodenal ulcer. The bacteriological examination leaves uncertain the source of the pathogenic bacteria to which experience leads us to attach importance, but the occurrence of a focal reaction after administration of an autogenous vaccine may settle the question, which may later receive complete confirmation from a successful therapeutic result. The chief value of bacteriological examinations of the stools lies, however, in the importance of the information they commonly furnish regarding acute and chronic infections of the colon.

For the collection and transmission to the Laboratory of suitable culture material, the stool specimen tubes described in Chapter V. are very convenient. The patient should be instructed to catch some of the last portion of a motion upon paper, to avoid its mixture with urine, and to fill only the wire loop with the material.

In the Laboratory, an emulsion of the specimen must be made in sterile normal salt solution. The proper density of this emulsion for cultural purposes is difficult to define, but, as a rule, water to the amount of from ten to twenty times the original volume should be used. It is generally necessary, in order to obtain discrete colonies, to make a further dilution on an agar surface in the way already described for sputum. From this surface the material should be distributed on agar and hæmoglobin agar tubes. It is absolutely essential that anaerobic cultures be made. The examination of direct films is less important than for sputum.

Occasionally we have to deal, not with a specimen of the stools, but with intestinal mucus. This should be thoroughly washed in several changes of sterile normal salt solution, and then treated in the same way as has been directed for sputum.

3. The Genito-Urinary Tract

As will readily be understood, the investigation of the flora of the genito-urinary tract requires the application of various

different methods, according to the subject to be examined, and the purpose in view.

In the male subject, the urine is best obtained, after external cleansing, as a mid-stream specimen, passed into a sterilized urine glass, or specimen tube. A specimen so obtained is practically free from external or urethral contamination. It is rarely necessary to procure a catheter specimen. About 10 c.c. of the urine should be put in a centrifuge tube and centrifuged for from ten to fifteen minutes. The fluid should thereafter be decanted into a clean test tube. Great care must be taken that the deposit is not lost, for it is by no means always firmly adherent to the bottom of the glass. It is best to allow the inverted tube to drain for a few minutes over a piece of sterilized filter paper. The deposit may be removed, either with a platinum loop or with a fine glass pipette, newly drawn out in the Bunsen flame. Before cultures are made, a film should be stained by the Gram-neutral red method, or other staining process, according to the purpose in view. Microscopical examination of these preparations enables us to determine if there is any acute or subacute inflammatory lesion of the urinary tract (indicated by an abundance of polymorphs), and at the same time gives us some idea of the number of bacteria present. Cultures must be made upon agar and hæmoglobin agar media in suitable dilutions. Anaerobic cultures must never be omitted. If there is a deposit of urates, the urine should be gently heated until they are dissolved. If the stay in the centrifuge is shortened to about five minutes, it is generally possible to get cells and bacteria deposited before solid urates are again formed. The urine decanted after centrifuging should be tested for albumen, etc.

In cases of urethritis, cultures should be made from the discharge, if it is available. If it is not, a small platinum loop passed about an inch up the urethra will generally provide sufficient culture material. In some cases of chronic urethritis a valuable plan is to get the patient to pass into a sterilized urine glass about 10 c.c. of the first portion of the morning urine. The micro-organisms that have been lying free in the urethra will be contained in this specimen, and they can easily be centrifuged out and examined in film and

culture. In cases of chronic prostatic infection we may depend for our cultures either upon prostatic threads contained in a specimen of urine, or employ the method of prostatic massage in order to procure a specimen of discharge. For the culture of the gonococcus it is best to use a +6 medium. It must always be borne in mind that in some cases of long-standing infection by this diplococcus, growths can be obtained only under anaerobic conditions.

In the female subject a specimen of urine satisfactory for bacteriological purposes can, as a rule, be obtained only by the use of the catheter. Intelligent patients may, however, be able to provide a mid-stream specimen, free from contamination. Cultures from the genital tract are chiefly required from the cervix and uterus. In many cases in which it is difficult or undesirable to procure ordinary swabs, satisfactory information can often be obtained by a bacteriological examination of the menstrual blood, a specimen of which the patient can provide as a swab taken from the diaper and plunged into an agar immersion tube.

4. The Central Nervous System

Most of the bacterial actions upon the central nervous system are exercised through toxins carried from foci of infection in the alimentary, respiratory or genito-urinary tracts. Infections of the central nervous system have to be investigated chiefly through a specimen of the cerebro-spinal fluid obtained by lumbar puncture.

Lumbar Puncture.—Special platinum-iridium needles, a little over three inches in length, are required, as steel needles inserted between the vertebræ are apt to be broken by a sudden movement of the patient. At least two needles should be provided for one case. Each must have inserted into it a strong wire, long enough to project beyond the point, and having a loop turned on the other end. Each needle, with the wire thus inserted, should be put in a test tube (plugged with cotton-wool) and sterilized in the autoclave. These test tubes must not be afterwards opened until the moment the needles are required for the operation. Two or

more centrifuge tubes, plugged with cotton-wool and sterilized in the autoclave, must also be provided.

The patient must be in bed. He may lie on his left side, or sit with his legs across the bed and the hips at the edge. The spine must be arched forwards. We wish to draw fluid from the dural sac, some distance below the point at which the spinal cord terminates. It has been found that it is best to puncture the sac through the space between the fourth and fifth lumbar vertebræ. The spine of the fourth lumbar vertebra lies in a straight line drawn between the highest points of the two iliac crests. This landmark having been found, the skin should be sterilized by means of iodine or other suitable antiseptic. A needle with the wire still in it, but drawn back from the point, is inserted half-an-inch below this mark, either a little to the right or the left side, and pushed forwards and slightly inwards. The ligamentum subflavum offers a little special resistance, which is, however, easily overcome. If bone is struck, the needle should be withdrawn a short way, and then directed a little farther up, or down. A three-inch needle generally requires to be pushed in nearly up to its brass head. In order to test if the dural sac has been entered, the wire should be first pushed in a little and then withdrawn. If the sac has been pierced, clear fluid will appear within a few seconds. The first few drops should be allowed to escape, and thereafter about 10 c.c. should be caught in a centrifuge tube. After the needle has been withdrawn, the wound is generally sealed with collodion.

For bacteriological purposes it is, as a rule, necessary to centrifuge the fluid and to make cultures from the deposit. The methods employed will vary with the purpose of the examination.

5. The Skin and Subcutaneous Tissues

Pus from boils and pustules does not, as a rule, require great dilution. The measures that it is necessary to take in cases of acne in order to obtain growths of the *bacillus acnes* are described in Chapter VI., section 7. An eczematous skin should be partially sterilized before cultures are made

from it. The important bacteria are in the tissues, and destruction of those on the surface facilitates the examination. An excellent method is to apply absolute alcohol for about a minute and to allow it to evaporate, and then to make cultures from scrapings taken with a sharp knife. It is often of advantage in such cases to employ dry cupping by means of a test tube heated at the closed end and then held firmly over the skin while the glass is cooling. Fluid is thus drawn from the lymph spaces, and, after the tube has been taken away, a loopful can be secured and spread on culture media.

6. Bones and Joints

Except in cases in which there are open wounds or sores, bacteriological examinations of diseased bones and joints can be made only in conjunction with surgical procedure. Whether the material is tissue or fluid, the cultural methods already described are likely to be applicable, although they will vary greatly with the probable nature of the infections. The importance of making anaerobic, as well as aerobic, cultures can hardly be too strongly insisted upon.

7. The Blood

From time to time it is necessary to make cultures from the blood. The method of obtaining it for bacteriological purposes is so well known that it hardly needs description here. The needle must not be too fine, and in order to prevent its being blocked by clot, it should be sterilized in vaseline raised to a temperature considerably above 100° C. It must be taken from the vaseline with a pair of suitable forceps and violently shaken in order to remove superfluous fluid from the tube. The interior will remain lined with a thin layer of vaseline, which prevents clotting. The blood, most conveniently taken from a vein of the arm, may be caught in a sterilized tube, or it may be run straight into a broth. Many cultural methods are applicable, but the most generally useful is probably that of mixing from 5 to 10 c.c. of blood with about 50 c.c. of Lemco-peptone broth. After incubation for twenty-four hours the broth is subcultured upon agar and hæmoglobin, or blood agar, surfaces.

CHAPTER VIII

THE PREPARATION OF STANDARDIZED BACTERIAL EMULSIONS

For the purposes of therapeutic immunization we require sterilized and accurately standardized bacterial emulsions of suitable strength. The method of standardizing by counting the bacteria, a description of which will be found in any of the systematic text-books of bacteriology, is, in my opinion, not sufficiently accurate. Standardization by the opacity of the emulsion, compared with that of a control of known strength, is, I think, open to the same objection. I would urge the abandonment of these methods, and the adoption of one or other of the two gravimetric methods here described.

The question of the kind of culture that should be used has given rise to some discussion. The first essential is that it should be a pure growth. Too much has been made of the importance of primary cultures. It is only occasionally that these are available as pure growths. The most common instances occur with cultures of staphylococci from boils and pustules, and of coliform bacilli from the urine in cases of cystitis. It is, however, probably important that the growth to be used should not have been frequently subcultured: the first subcultures should be used, if possible. It has been alleged that bacteria grown upon human blood media are alone satisfactory for the preparation of vaccines. It is, however, far from having been proved that such cultures present any advantage, whilst there is overwhelming evidence that cultures made on agar and hæmoglobin agar, and in serum broth, are capable of giving results that leave nothing to be desired.

Various methods have been recommended for killing the bacteria in the emulsions to be used for therapeutic purposes. Heat, various antiseptics and ultra-violet rays are available. There is some evidence to show that heat, even though not

exceeding 60° C., may occasionally injure a vaccine. The means to which there seems to be least objection is sterilization in 0·5%, or 1% carbolic acid in water. The stronger solution will kill most bacteria, in an emulsion of average strength, within twelve hours.

The first of the two gravimetric methods described below has been used in the Laboratory of the Scottish Asylums since 1912. The second has recently been worked out by Mr J. J. Ritchie, late Senior Assistant in the Laboratory, and presents some advantages. It will, however, be found rather more difficult to carry out than the other, requiring special skill in the use of the chemical balance.

The following special apparatus is required :—

A chemical balance, weighing to a 0 1 fraction of a milligram.
Centrifuge tubes, plugged and sterilized.
Test tubes, $5 \times \frac{5}{8}$ in., plugged and sterilized.
Test tubes, $4 \times \frac{1}{2}$ in., plugged and sterilized.
A simple apparatus for drying glass tubes by heat.
A hot-air oven.
A calcium chloride desiccator.
Glass rods, about 12 in. long and about $\frac{1}{8}$ in. thick, with one end rounded in the Bunsen flame.
A sheet of tinfoil.
A 1 c.c. graduated pipette.

A stock of the following must also be kept in tubes or flasks :—

Sterilized distilled water.
Sterilized normal salt solution.
5% carbolic acid in water.
$\frac{1}{4}$% carbolic acid in normal salt solution.

1. The Gravimetric Method of Standardizing Bacterial Emulsions

Either surface or broth cultures may be used. If the culture is a surface one, put about 5 c.c. of 1% carbolic acid into a centrifuge tube. Scrape off the growth with a platinum loop and emulsify it in the fluid. If approximately more than 10 mg. of vaccine are being prepared, a proportionately larger quantity of carbolic acid solution must be used. Carefully label the tube and place it in the incubator

at 37° C. overnight. If a broth culture is employed, place a sufficient quantity in a centrifuge tube, and centrifuge it in the electric centrifuge until the micro-organisms are deposited. Five to ten minutes generally suffice. Decant the fluid. If the broth has been a serum one, wash the deposit with sterile normal salt solution and centrifuge again. Pour off the fluid and replace it by 1% carbolic acid solution. Put the tube in the incubator at 37° C. overnight. From this point the method is the same, whether a broth culture or a surface one has been employed. After from twelve to twenty-four hours have been allowed for sterilization, centrifuge the emulsion for from fifteen to twenty minutes. Pour off the fluid, being careful that the deposit is not lost. Allow the tube to stand for a few minutes inverted over a piece of filter paper (sterilized over the Bunsen flame). Test for sterility by spreading a little of the deposit upon a lactose agar or hæmoglobin agar surface (set up anaerobically if the bacterium is an anaerobic one). Sterilize a disc of filter paper and fold it over the mouth of the centrifuge tube, fixing it in position with a small elastic band. Put both tubes together in the incubator. Next day, note if the vaccine has passed the test for sterility. If the deposit is dry, proceed with the weighing, or put the tube in the desiccator until it is required.

Fixed to the upright supporting one pan of the chemical balance, it is necessary to have a wire or other attachment by means of which a centrifuge tube can be held erect in the centre of the pan. Sterilize in a Bunsen flame from four to six inches of one end of a glass rod and set it aside to cool. After having removed the paper cap, place the centrifuge tube in position. Counterpoise it exactly with sand or weights placed in the other pan. Sand is very convenient for this purpose, but there are some objections to its use. It is probably better to use weights and to complete the counterpoising by means of minute scraps of tinfoil. Take the tube from the balance and pour into it about 1 c.c. of distilled water. With the aid of the sterilized end of the glass rod, which will now have cooled, detach the vaccine material from the bottom of the tube. This grinding process should not be prolonged, as it is much easier than is probably believed to

make an opaque emulsion of ground glass. It is best simply to see that the whole of the vaccine is detached from the tube, and that no very large particles remain floating in the fluid. The emulsification will be completed later by shaking. Pour the emulsion into a sterilized tube ($5 \times \frac{5}{8}$ in.). Wash out any remaining particles by means of about 1 c.c. of distilled water, and add this to the emulsion already in the test tube. Next, place the centrifuge tube in the drying apparatus, over a Bunsen flame. The heat must not be so great as to char the label. When all water has been driven off, place the centrifuge tube in the desiccator. After it has had sufficient time to cool, proceed with the second weighing. Place it, as before, in the pan of the balance. It will be found that there has been a certain loss of weight. This loss must be accurately estimated by means of the rider, and, if necessary, by weights put in the pan. Register on the label of the test tube containing the emulsion the weight of the vaccine thus ascertained. Pour the emulsion into a sterilized cubic centimetre measure, and, with $\frac{1}{4}\%$ carbolic acid in normal salt solution, make up the amount of fluid to the same figure in cubic centimetres as the weight of the vaccine in milligrams. Thus an emulsion of the strength 1 c.c. = 1 mg. will be obtained. Label the tube carefully, stating the patient's name, the nature of the vaccine, its strength and the date of the completion of its preparation. Heat a little hard paraffin in a porcelain dish, over a Bunsen flame, until vapours rise from it. Dip the lower end of the cotton-wool plug in the melted paraffin and immediately replace it in the test tube. When the paraffin has cooled, the tube may be regarded as sealed. Shake the tube vigorously, holding a finger on the stopper as a precaution against its being forced out. The vaccine is now ready for further dilution, and for tubing, or bottling, for use, in the strength required. If it is thought necessary to have it in normal salt solution and that it should contain exactly $\frac{1}{4}\%$ carbolic acid, an equal volume of $\frac{1}{2}\%$ carbolic in $1\frac{1}{2}\%$ salt solution must be added to the emulsion in distilled water, before it is standardized to the strength of 1 c.c. = 1 mg.

If the vaccine is found not to be sterile, proceed with the weighing, and add carbolic acid to the emulsion up to $\frac{1}{2}\%$. After some hours, test again. In all cases in which a vaccine

fails to pass the test for sterility, the possibility of *bacillus subtilis* contamination must be considered. Examine a film of the growth, stained by Gram's method. *Bacillus subtilis* is easily recognized by its spores. A vaccine contaminated by this bacterium cannot be sterilized and must be put away.

2. The Unit Gravimetric Method of Standardizing Bacterial Emulsions (J. J. Ritchie)

In this method a unit of known volume of the vaccine emulsion is dried and weighed, and from the result the weight of dried bacteria in the major portion of the emulsion is then calculated.

(1) Prepare an emulsion of the micro-organism in distilled water, using generally from five to ten cubic centimetres. If a broth culture is used, centrifuge it, decant the fluid, and add distilled water to the deposit. Plug the tube with melted hard paraffin in the usual way and shake it vigorously in order to obtain a uniform suspension of bacteria.

(2) Take a clean test tube, of the size $4 \times \frac{1}{2}$ in., and attach to its neck a piece of floral wire, leaving a loop by means of which it can be hung on the hook of the chemical balance. Sterilize a 1 c.c. graduated pipette (a throttled one is to be preferred) by immersion in lysol solution. Dry the interior by passing alcohol through it, and then ether. Take up exactly 1 c.c. of the emulsion and put it in the test tube. Place this in the hot-air oven at 105 to 110° C. to dry. About an hour is generally required. From this point, handle the tube only with forceps. Avoid touching it with the fingers. Remove the dry tube from the oven and place it in the calcium chloride desiccator, where it must remain until cool. Pour the other portion of the emulsion into a graduated tube and note the exact volume. Return it to the original tube. Put into the measure exactly one-fifth of the volume of 5% carbolic acid, shake it up, and add it to the tube containing the emulsion. Leave the tube in the incubator overnight.

(3) Find the weight of dried bacteria in the unit volume. Take the tube from the desiccator and counterbalance it in the chemical balance, using weights and fragments of tinfoil. Remove bacterial deposit by washing out with distilled water

and gentle rubbing with a clean glass rod. Dry the tube again in the oven and allow it to cool in the desiccator. Replace the tube in the balance and find the loss of weight in milligrams. This figure represents the weight of bacteria in 1 c.c. of the emulsion, and the same figure multiplied by the number of cubic centimetres in the remaining portion of the emulsion (already noted) will indicate the weight of bacteria in it.

(4) After twenty-four hours, test the emulsion for sterility. By addition of $\frac{1}{4}\%$ carbolic acid in normal salt solution dilute the emulsion down to a definite strength. As a rule this should be 1 c.c. =1 mg. The vaccine is not ready for further dilution and use until it has passed the test for sterility.

It will be observed that in this method the emulsion is made in distilled water. If it had been necessary to make it in normal salt solution, the method would have been impracticable. There are, however, the strongest reasons for believing that the disintegrating action of distilled water upon the bacteria, instead of being injurious, renders the vaccine more suitable for the therapeutic immunization.

The following is an example of an actual weighing. An emulsion of *staphylococcus pyogenes aureus* was prepared in distilled water. 1 c.c. of this emulsion was placed in a tube and dried. The deposit was found to weigh 2·5 mg. The remaining portion of the emulsion measured 6·5 c.c. The weight of the bacteria in it was therefore (6·5 × 2·5) 16·25 mg.

Sensitized vaccines (see Chapter IV. for a criticism of their value) may be prepared by placing the living bacteria in from 5 to 10 c.c. of the corresponding anti-serum for twelve to twenty-four hours at 37° C. Thereafter the bacteria must be centrifuged out, washed with normal salt solution or distilled water, and sterilized and standardized by one or other of the two methods just described.

Standardization of Simple and Compound Bacterial Emulsions for Use.—As a rule, bacterial emulsions to be used for therapeutic purposes should be made of such a strength that 0·1 c.c. contains the ordinary initial dose. The strength should be stated on the label as the fraction of a milligram in one cubic centimetre. Thus, an emulsion of *streptococcus*

pyogenes might be made of the strength 1 c.c. = 0·1 mg. The initial dose of 0·1 c.c. would contain 0·01 mg. The ordinary initial and maximum doses of the various important pathogenic bacteria are given in Chapter X.

A supply of from 10 to 20 c.c. of a simple bacterial emulsion is generally needed for the treatment of a case. The stock emulsion of the strength 1 c.c. = 1 mg. must be diluted to the extent required. In order to make up 20 c.c. of an emulsion of *streptococcus pyogenes* of the strength 1 c.c. = 0·1 mg., 2 mg. of dried bacteria are required. This amount is contained in 2 c.c. of the stock emulsion. Therefore we pour 2 c.c. of the stock emulsion into a graduated tube and add ¼% carbolic acid in normal salt solution up to 20 c.c. More dilute emulsions are made in a similar way. Thus it may be necessary to make a pneumococcus vaccine of the strength 1 c.c. = 0·0005 mg. The most accurate method is to make successive 1 in 10 dilutions, commencing with the standard stock emulsion—namely, of the strengths 1 c.c. = 0·1 mg., 1 c.c. = 0·01 mg., and 1 c.c. = 0·001 mg. If 10 c.c. of the last emulsion are diluted with an equal volume of normal salt solution, 20 c.c. of the strength 1 c.c. = 0·0005 mg. will be obtained. The calculations are really extremely simple, and, once the method is understood, they should present no difficulty.

The preparation of compound emulsions, perplexing as it may appear on first view, is really equally simple. The same rule of having ten times the initial dose of each element in 1 c.c. should be followed. When we know the ordinary initial dose of each element, and have fixed upon the total volume of the compound emulsion, the rest is easy. We have merely to calculate the weight of each element required to measure out the corresponding volume of each element, and finally to make up the full amount with ¼% carbolic acid in normal salt solution. The following examples should serve to make the method quite clear :—

1. **20 c.c. of Compound Catarrhal Vaccine.**

1 c.c. =	*Bacillus influenzæ* . . .	0.1 mg.	×20=2 mg.
	Micrococcus catarrhalis . .	0·2 mg.	×20=4 mg.
	Pneumococcus . . .	0·05 mg.	×20=1 mg.
	Streptococcus pyogenes . .	0·05 mg.	×20=1 mg.

Four tubes containing the respective emulsions of the strength 1 c.c. =1 mg. are at hand. A graduated tube is taken and the emulsions are added to it thus: 2 c.c. of the bacillus of influenza, 4 of the *micrococcus catarrhalis*, 1 c.c. of the pneumococcus, and 1 c.c. of *streptococcus pyogenes*. The tube now contains 8 c.c.; 12 c.c. of $\frac{1}{4}\%$ carbolic acid in normal salt solution must be added to complete the preparation of the desired emulsion.

2. 30 c.c. of Compound Vaccine for Rheumatoid Arthritis.

1 c.c.=	*Staphylococcus pyogenes aureus*	0·5	mg.	×30=	15 mg.
	Streptococcus anginosus . .	0·1	mg.	×30=	3 mg.
	Streptococcus pyogenes . .	0·1	mg.	×30=	3 mg.
	Pneumococcus (from cases of rheumatoid arthritis) . .	0·005	mg.	×30=	0·15 mg.

In addition to the ordinary stock emulsion of the first three, it is necessary to provide a 1 in 10 dilution of the corresponding pneumococcus emulsion—that is to say, of the strength 1 c.c. =0·1 mg. To make up the compound emulsion we take 15 c.c. of the *staphylococcus pyogenes aureus* emulsion, 3 c.c. of *streptococcus anginosus*, 3 c.c. of *streptococcus pyogenes*, and 1·5 c.c. of the diluted emulsion of pneumococcus. The resulting volume of 22·5 c.c. is made up to 30 c.c. with $\frac{1}{4}\%$ carbolic acid in normal salt solution.

3. 15 c.c. Staphylococcus M.N.F. and Bacillus acnes Vaccine.

1 c.c.=	Staphylococcus M.N.F. . . .	1	mg.	×15=	15 mg.
	Bacillus acnes . . .	0.5	mg.	×15=	7·5 mg.

In this instance a staphylococcus emulsion of at least double the ordinary strength is required. Indeed, the dose of this bacterium being comparatively high, it is customary to make the stock emulsion of the strength 1 c.c. = 2 mg. Of this 7·5 mg. will give the required 15 mg., and the addition of 7·5 c.c. of the ordinary *bacillus acnes* emulsion, of the strength 1 c.c. =1 mg., will complete the preparation of the desired double vaccine.

It is perhaps necessary to make it clear that when two or

more vaccines are being given in a case, it is not essential to combine them in one emulsion. Indeed, the rule should be to have each element in a simple emulsion, and to regulate the respective doses in accordance with the principles laid down in the next chapter.

Bottling and Tubing.—Of the many devices that have been tried for the convenient preservation of immunizing emulsions for actual use, I think the best is the glass-stoppered bottle of 20 c.c. capacity. Its shape does not matter very much, but it is of importance that the neck should be short. Stoppered bottles of much smaller capacity, as well as larger ones, are also useful.

These bottles must be carefully cleaned, and plugged with cotton-wool. A stopper that has been found to fit accurately should be tied with string to the neck of each. Sterilization must be carried out in the autoclave. A dozen or more bottles should be prepared at a time and put aside in a clean dust-proof box. When a bottle is to be filled with vaccine, the cotton-wool stopper is taken out ; the glass stopper is detached from the neck and its lower end is sterilized in the Bunsen flame. After a few seconds have been allowed for cooling it is placed in the mouth of the bottle and retested with regard to its being a proper fit. If everything about the bottle seems satisfactory, the emulsion that has been prepared is poured into it and the stopper firmly fixed. A piece of tinfoil of suitable size is then folded over the stopper and neck of the bottle. The tinfoil may be conveniently sterilized in 10% formalin in alcohol, or in 5% carbolic acid in alcohol.

Vaccines may also be conveniently put up in sterilized specimen tubes of 1·5 c.c. capacity. The tubes must be carefully cleaned and packed, without corks or plugs, into test tubes of the size 6×1 in. These must be plugged with cotton-wool. Sterilization is then carried out in the autoclave. A block of wood, with twelve shallow holes of suitable size, is very convenient for holding the tubes when they are being filled. Corks of suitable size are sterilized in a porcelain dish containing hard paraffin, heated to fuming point by means of a Bunsen flame. About 1 c.c. of the emulsion should be put into each tube. With forceps, the points of

which have been sterilized by heat, corks are taken from the hot paraffin and inserted into the mouth of each tube. When the paraffin on the corks has set, the upper end of each tube should be momentarily dipped into the hot paraffin.

Labelling.—Accurate labelling is of the utmost importance. Gummed labels of various sizes should be kept in stock. A simple vaccine should be labelled thus:

Mr John Smith
20 c.c. autogenous vaccine
Staphylococcus pyogenes aureus
1 c.c.=0·5 mg.
Dose—0·1 c.c. to 1 c.c., or more
15th April, 1920. W. F R.

The following are two examples of the way in which it is recommended compound vaccines should be labelled :—

Miss White
20 c.c. autogenous vaccine

1 c.c.=	*Streptococcus pyogenes* . . .	0·1 mg.
	Streptococcus anginosus . . .	0·1 mg.
	Micrococcus catarrhalis . . .	0·2 mg.

Dose—0·1 c.c. to 2 c.c.
13th April, 1920. W. F. R.

Mr H. Black
20 c.c. autogenous vaccine

1 c.c.=	*Staphylococcus pyogenes aureus* . .	0·5 mg.
	Bacillus proteus vulgaris . . .	0·1 mg.

Dose—0·1 c.c. to 1 c.c., or more
26th March, 1920. W. F. R.

Storage of Vaccines.—Some system of storing the large number of stock vaccines belonging to various cases must be adopted in any laboratory in which work for the purposes of therapeutic immunization is carried out. In the Laboratory of the Scottish Asylums the stock tubes are kept in wire holders, each of which has forty-eight holes, arranged as four rows of twelve. Some twelve of these holders are placed lengthwise on the shelves of a cupboard. Four holes—that is, one row from front to back—are apportioned to the tubes

belonging to each case (in some instances two rows are required), and the cases are arranged alphabetically. All vaccines more than eighteen months old are put out.

Keeping Properties of Vaccines.—If vaccines are kept in sealed tubes their properties remain unimpaired for a year, or longer. If air is admitted they seem to deteriorate with some rapidity. It is therefore important that all stocks kept in tubes should be "paraffin-plugged." The verb is clumsy, but its use, in the laboratory at least, is inevitable.

CHAPTER IX

THE PRACTICE OF THERAPEUTIC IMMUNIZATION

THEORETICAL considerations and the lessons taught by experience are the foundations of the rules here laid down. The former have already been specially dealt with in some detail in Chapters III. and IV.

A case has been made the subject of bacteriological investigation in the laboratory, autogenous vaccines have been prepared, and we are ready to apply our therapeutic agents. We must understand the method of using them, and know something of the immediate and remote effects they are likely to produce upon the patient.

Technique of Injection.—The only way in which vaccines should be used is, in my opinion, by simple hypodermic injection. Intra-venous injection has been tried and strongly advocated, but it has serious disadvantages and risks on account of which, I think, the method should not be used.

The syringe should be one of 2 c.c. capacity, with glass barrel, permitting of the contents being seen. Only syringes marked in cubic centimetres, and 0·1 division of a centimetre, should be used. The needles should be as fine as possible, consistent with the easy passage of the emulsion through them. Great care must be taken to see that they are sharp and especially that the point is not turned, as it is very apt to become through having accidentally been made to strike the side of the vaccine bottle. Before the bacterial emulsion is taken up, the syringe, with needle firmly attached, must be made thoroughly aseptic. This should never be done by boiling the instrument, but by means of a suitable antiseptic. I use 5% carbolic acid in alcohol, both for the syringe and for the skin. About 0·2 c.c. of this or other suitable antiseptic should be drawn into the syringe. The instrument should then be held with the needle pointing upwards, and the piston drawn out to its full length. After a

few seconds the piston should be pushed up to the top of the barrel, and the antiseptic as it is ejected should be allowed to run down the outside of the needle. Thereafter, the needle must not be touched with the fingers. Before the emulsion to be injected is taken up, the remains of the antiseptic must be got rid of. Sterile normal salt solution, with $\frac{1}{4}\%$ carbolic acid added, may be kept at hand and used for this purpose, but, as a rule, it is convenient to remove the antiseptic by means of a little of the vaccine emulsion. About 0·2 c.c. of this should be drawn into the syringe and ejected again in the same way as has been recommended should be done with the antiseptic. The exact quantity of emulsion it is desired to inject, or of each emulsion, when several are being given, should then be carefully drawn up into the instrument.

Opinions differ greatly as to the best place at which to make the injection. Two considerations must have chief weight, that of convenience and that of obtaining fresh ground for each injection. In my opinion, the best site is the back of the upper arm from two inches above the point of the elbow to the same distance below the top of the shoulder. The left and right arm should be taken alternately, and spots at which injections have previously been made should, as far as possible, be avoided. The point selected must be made aseptic by applying a suitable antiseptic. As already indicated, I use 5% carbolic acid in alcohol. It serves to sterilize the surface in from ten to twenty seconds. It must not be allowed to act for more than three minutes. The skin at the spot should be pinched firmly between the thumb and forefinger, and the needle pushed quickly through the skin into the subcutaneous tissues. I think it is a mistake to inject into muscles. The operation may be made painless by the previous application of an ethyl chloride spray. This should be allowed to act intermittently upon the spot at which the injection is to be made for about thirty seconds. Its action should never be pushed to actual freezing of the tissues. Chilling is all that is required to produce anæsthesia.

Dosage.—The dosage of the various vaccines commonly used is now as definitely established as that of drugs. For each there is a normal initial dose and a normal maximum. For example, the initial dose of *staphylococcus pyogenes* is

0·05 mg., of *bacillus coli communis* and *streptococcus pyogenes* 0·01 mg., of the pneumococcus of rheumatoid arthritis 0·0001 mg., and of the tubercle bacillus 0·00001 mg. The maximum dose, to be reached only after numerous gradually increased doses have been given, is generally about twenty times the initial dose. The ordinary dosage of each pathogenic species is considered in Chapter X.

Intervals between the Doses.—In the early stages of therapeutic immunization, in cases of chronic infection, if the focal reactions are not severe, an interval of three or four clear days is sufficient. When the doses have been raised, or if focal reactions have been severe, an interval of one week is probably the most suitable. Occasionally, towards the end of a course of immunization, when high doses have been reached, it is a good plan to extend the interval to a fortnight, or even three weeks. In acute infections, small doses should be given every day, or every second day.

Phenomena following the Injection of a Vaccine. Local, Focal and General Reactions.—The injection of a suitable amount of vaccine is followed by certain reactive phenomena with which it is of the utmost importance to be acquainted, because some of them are our chief guides to correct dosage.

We have to distinguish between *local*, *focal* and *general reaction*. The *local reaction* is the inflammatory action that occurs at the point of injection. As a rule it is slight, showing itself as a little swelling, congestion and tenderness on the following day. The intensity of the local reaction is of no value as a guide to dosage. Some vaccines produce more local irritation than others. *Micrococcus catarrhalis* and gonococcus vaccines are notorious in this respect, generally causing much swelling and congestion, as well as tenderness, beginning a few hours after the injection has been made and lasting for two days, or longer. Such disturbances are, however, chiefly caused by the early doses, although occasionally "a bad arm" may follow one of the later injections, especially if the dose is pushed up too rapidly.

The *focal reaction* is an active congestion in the infective focus. It occurs, or at least is perceptible, only in cases of chronic infection. It is erroneous to speak of a focal reaction to a vaccine in a case of acute infection, because in the infec-

tive focus there are already established all the features of a focal reaction. In chronic infections the focal reaction begins, as a rule, about twelve hours after the injection has been given, and lasts from twelve to thirty-six hours. In the respiratory tract, it is commonly attended by local irritation, increased secretion and general aggravation of the ordinary symptoms of the malady. In infections of the conjunctiva, the reaction becomes visible on account of the general capillary congestion. In skin infections, evidence of a focal reaction is usually quite distinct; for example, indolent acne pustules become intensely congested and, through attraction of polymorphs to them, tend to ripen rapidly. The process is really a reparative one, although it is apt to be interpreted as a mere aggravation of the malady. In some cases of chronic infection of the pharynx, the occurrence of a focal reaction may be observed in the intense congestion of the tissues. As a rule, however, we have to depend upon the patient's description of his sensations in order to estimate the intensity of the focal reaction. In regard to this matter some patients are intelligent and extremely helpful, but, unfortunately, others are just the reverse. In cases of chronic infection of the lower respiratory tract, for example, the patient will generally report temporary increase of cough and expectoration. In cases of chronic infection of the intestinal tract, there will commonly be reported the recurrence of some disagreeable sensations, or experiences that are characteristic of the malady, such as localized pain, sickness, loss of appetite or an attack of diarrhœa. In cases of chronic cystitis the patient experiences a miniature attack of the malady. In chronic urethral infections, there is transient increase of discharge and other signs of local congestion.

By the *general reaction* is implied disturbances that are localized neither in the seat of injection nor in the infective focus. They are essentially due to a general toxic action, resulting from overflow of toxin from the infective focus, or, more rarely, owing to the excessive amount of toxin injected as vaccine. Rise of temperature rarely occurs, except when an excessive dose has been given. Distinct and even violent focal reactions may occur without disturbance of temperature. The general sensations of which the patient commonly

complains are malaise, headache, sickness and drowsiness. With properly regulated doses, these symptoms are trivial in degree.

Therapeutic immunization should be begun with the initial dose that experience has shown to be generally suitable. Thereafter, the dose must be increased or diminished according to the amount of focal and general reaction produced by the previous one, and under the guidance of the lessons of the accumulated experience of the immunizer. The best results are obtained by the repetition of a series of mild focal reactions. To obtain these the dose usually requires to be increased from week to week. One violent focal reaction may do good, but it is certainly injurious to push the dose in such a way as to cause a series of violent reactions. If the ordinary initial dose reveals hypersensitiveness, it is best to have the emulsion diluted to one-tenth of its original strength, and to resume with a dose of one-tenth of that previously given.

Examples of extreme hypersensitiveness to vaccines are quite common. They have been observed especially in cases of infection by pneumococci, diphtheroid bacilli, *staphylococcus pyogenes*, *bacillus influenzæ*, and the tubercle bacillus. In these instances the focal and general reactions are very severe and prolonged for several days.

It is sometimes difficult to distinguish between the usual symptoms of the malady and the effects of an excessive dose of vaccine. It is to be remembered that natural invasion by bacteria from an infective focus, an excessive dose of autogenous vaccine and a chill may each be followed by the liberation of exactly the same amount of toxin in the infective focus. When this fact is realized, it may be understood how difficult it sometimes is to distinguish which of the three is the cause of a "flare up." When, in consequence of a chill or other cause, there has been a rapid multiplication of bacteria in an infective focus and their extension to adjacent areas, it is inevitable that there should be severe and prolonged toxic disturbances, because, even if the invasion is checked, the gradual destruction of the bacteria must result in the liberation of a large quantity of toxin.

An essentially different kind of reaction, which is really a local toxic action and not a focal reaction at all, may be

observed especially in rheumatoid arthritis and in some forms of nervous disorder. It is due to the circulation of a special bacterial toxin in the blood, and its fixation in a tissue that has an affinity for it. In rheumatoid arthritis of pneumococcal origin, an excessive dose seems to be followed by an increase of the special toxin in the blood about forty-eight hours after the vaccine has been given, with consequent increase of pain in the affected joints. If excessive doses are repeated, this increase of pain is apt to become continuous. In nervous disorders, such as some common types of neurasthenia, exophthalmic goitre, and some forms of insanity, all dependent upon the neurotoxic action of aerobic or anaerobic diphtheroid bacilli, excessive doses are followed within a few hours by aggravation of the characteristic nervous symptoms. This is almost certainly due to increase of the special toxins in the blood on account of their liberation within the infective focus, and their fixation in the nervous tissues that have an affinity for them.

In all cases of chronic bacterial infection in which we carry out therapeutic immunization, the patient exhibits, at least theoretically and in most cases actually, two phases in his reactivity to the vaccine: (1) that of hypersensitiveness, and (2) that of tolerance. In the first, we have to break down the patient's sensitiveness to the bacterial toxin, a condition that is dependent upon the presence of the corresponding bacteria in his tissues. In the second, the patient no longer harbours the special bacteria in his tissues, and therefore he experiences no focal reactions. His tolerance of the bacterial toxin at this stage is often surprising, and it should be taken advantage of to the full. By pushing the dose too much we may, however, produce severe general reactions, such as occur in the course of protective inoculation against the typhoid bacillus, or against the influenza bacillus. An endeavour should be made to establish in the patient a high degree of tolerance for the toxins of the bacteria against which he has been fighting. This is the surest way to prevent a relapse. As a rule, from twenty to forty times the initial dose of the vaccine is sufficient, but I think there are cases in which we might with advantage carry the final dose to a much higher figure.

Fear of anaphylaxis in the practice of therapeutic immunization should be entirely dismissed. As has already been contended in Chapter IV., it is founded on erroneous ideas. In all cases in which we practise therapeutic immunization, the patient has already had either a prolonged and repeated experience of the toxin we are going to inject, or so short an experience that the anaphylactic state has not had time to develop. Moreover, in the latter case, the toxic action is continuous, and not intermittent, as is required for the development of this state. There is therefore no possibility of the occurrence of anaphylaxis. As a matter of fact, its phenomena are never seen in the course of therapeutic immunization. The effects of gross over-dosage have, however, frequently been erroneously interpretated as of this nature. Theories of anaphylaxis are not required to explain such toxic disturbances any more than they are needed to explain the action of any common poison.

As is well known, there was a stage in the history of therapeutic immunization when it was taught, and generally believed, that dosage could be properly regulated only under the guidance of the opsonic index. Opsonins compose only one of several specific anti-bacterial substances that are developed in the body in response to bacterial attack. There are also bacteriolysins, agglutinins and precipitins. Moreover, a much more important factor than the amount of opsonin in the blood, of which alone the test gives us any approximate estimate, is the number of phagocytes that reach the infective focus. A high opsonic index in the blood would be of little value if few phagocytes reached the infected area, whilst a comparatively low index might suffice if a larger proportion of phagocytic cells were available. Of these differences the opsonic index takes no account. These objections to attaching much importance to estimations of the opsonic index are, however, trivial in comparison with others that can be stated.

Even if the results of opsonic estimations had all the value that has been claimed for them, and if they could be made accurate and in accord with the conditions existing at the moment of injection of the next vaccine dose, the possibility of which must be denied, the time and labour required in

order to obtain the requisite information are so great that, if it were essential to use the opsonic index as a guide, therapeutic immunization could be carried out only in a very small number of cases. The application of the procedure to cases in which the infections are multiple (the rule) would not only be extremely laborious, but virtually impossible.

To be added to these objections is the fact that the true guide to dosage is the focal reaction, the intensity of which is not dependent upon opsonic actions alone, but upon a combination of all the specific anti-bacterial actions, only some of which are as yet known. To substitute for this a conventional and laborious estimation of one among the many specific anti-bacterial factors is to make a useless expenditure of labour that actually serves to obscure the important facts and to lead us away from the true principles that ought to guide us in the practice of therapeutic immunization.

The Use of Stock Vaccines.—There can be no question that much has been done in recent years to bring therapeutic immunization into discredit by the random use of stock vaccines by doctors who have been too ready to shoot a bow at a venture and foolish enough to blame the weapon instead of themselves for their failure to realize their extravagant expectations. Even autogenous vaccines in the hands of those who do not know how to use them can rarely do anything but harm. Nevertheless, stock vaccines have their rational use. There are cases in which it is impossible, and others in which it is unnecessary, for the patient to be at the expense of having a bacteriological investigation made and autogenous vaccines prepared. The limits within which the employment of stock vaccines is legitimate will, I think, appear in the course of the discussions in Chapter XI., in which also the composition of various useful compound stock vaccines will be given.

CHAPTER X

BACTERIAL INFECTIONS AMENABLE TO TREATMENT BY THERAPEUTIC IMMUNIZATION

In this chapter, each bacterial species is considered in regard to its special pathogenic action and to the application of therapeutic immunization to its suppression as a pathogenic agent. The same order is followed as in Chapter VI., to which the reader is referred for information regarding the position of each species in the group classification, its distinctive features and the methods by which it may be cultivated.

1. Staphylococci

Staphylococcus pyogenes albus. Staphylococcus pyogenes aureus.—These two types differ so little from each other in their pathogenic actions that they may be considered together. Both are common infective agents in the skin and subcutaneous tissues, and in the mucous membranes and deeper tissues of the alimentary, respiratory and genito-urinary tracts. They have also been found in the blood, generally as terminal infections.

In the skin and subcutaneous tissues they are the common causes of boils and carbuncles. In these, *aureus* is much the more common.

Staphylococcus pyogenes also commonly plays a part in the causation of various chronic forms of dermatitis, including eczema, acne and seborrhœa. In many cases of acne, however, only mannite non-fermenting staphylococci can be found. *Staphylococcus pyogenes* is also a frequent agent in the infection of wounds. Whitlows are commonly due to the action of a virulent strain that has been introduced through a wound in the skin of the finger, often too small to have attracted attention.

Chronic infections of the nasal and post-nasal passages

by *staphylococcus pyogenes* are of common occurrence and are important causes of chronic catarrh. Any of the accessory sinuses may be involved. Extension to the Eustachian tube is common. Similar infections of the lower respiratory tract are comparatively rare, and when they occur are almost always associated with other more important infections.

Staphylococcus pyogenes infections of the mouth are not common and only rarely important, the field being here left mainly to streptococci, Gram-negative diplococci and diphtheroid bacilli. *Staphylococcus pyogenes* occurs with considerable frequency in the stools in disorders of the intestinal tract, but generally in association with more important pathogenic bacteria.

In the male, *staphylococcus pyogenes* may be a primary cause of a more or less severe form of acute or chronic urethritis. Several cases of the kind have come under my observation. It may also accompany, or follow, gonococcus infections.

Therapeutic immunization is generally very successful. The initial dose is 0·05 mg. Hypersensitive cases are occasionally met with, and in these it is imperative to reduce the dose to one-tenth, in accordance with the rule already laid down. Intense malaise and prostration are the main symptoms complained of by such patients. The interval between the doses should at first be from three to four days, but it should gradually be extended, as tolerance of the toxin permits of increase in the amount. A tolerance of 0·5 mg. can generally be reached in from eight to ten weeks. In some cases, a tolerance of 1 mg. is attainable.

Staphylococci of the Mannite Non-fermenting Group.— Members of this group are common saprophytes, but, like *bacillus coli communis*, they occasionally invade the tissues and exercise an important pathogenic action. They are among the commonest causes of chronic inflammatory lesions of the skin, nasal passages and lower portion of the genito-urinary tract. In all of these situations, they are very frequently associated with bacilli of the diphtheroid group. The staphylococcus in acne pustules is generally of this kind. I have studied many cases of acne in which repeated endeavours to isolate *staphylococcus pyogenes* have failed.

Bacillus acnes, with which it is associated in this malady, is a diphtheroid bacillus.

Chronic infection of the nasal passages by these staphylococci is exceedingly common. Chronic urethritis, persisting after the disappearance of gonococci, is most frequently due to a double infection by mannite non-fermenting staphylococci and diphtheroid bacilli.

Warren Crowe (*Brit. Med. Journ.*, 27th Nov. 1920) contends that a variety of *staphylococcus epidermidis albus*, which must be identified with the staphylococci under consideration, is the bacterial cause of rheumatoid arthritis, and he supports his view by records of many cases of the disease successfully treated with vaccine. I have for many years had under consideration the possibility of a staphylococcus of this kind being an important factor in the causation of rheumatoid arthritis, and I believe that the evidence compels the conclusion that it is never more than an occasional secondary infection of very minor importance. The infection is in no way specially associated with rheumatoid arthritis. The beneficial action of the vaccine has an explanation different from that attributed to it. It is similar to that of *staphylococcus pyogenes* in the same disease. There is not the smallest ground for believing that *staphylococcus pyogenes* has any essential part in the causation of rheumatoid arthritis, and yet it is well known that a stock vaccine prepared from it benefits many cases. The beneficial action of a vaccine prepared from a mannite non-fermenting type of staphylococcus probably results in the same way. It is a pharmacological action of the staphylococcus group that is being exploited, and not a specific immunizing action. This does not, however, diminish the practical value of staphylococcus vaccines in the treatment of rheumatoid arthritis.

Therapeutic immunization against this group of staphylococci is very successful, excepting in cases of old standing diffuse dermatitis, in which only a measure of improvement can generally be claimed after prolonged treatment. The dose requires to be larger than that of *staphylococcus pyogenes*. A tolerance of 2 mg., and more, may often be attained.

2. Streptococci

As agents in the causation of common maladies, the streptococci far outweigh in importance any other bacterial group. This fact must be evident to anyone who considers the number of species and sub-species of pathogenic bacteria they include, and the frequency of the occurrence of the maladies to which infection by one or other of them is now known to give rise.

Streptococcus pyogenes. — This includes numerous sub-species, not yet clearly separated from each other by any cultural or morphological character, but sharply distinguished by their pathogenic actions. Thus, there must be an essential difference between the streptococcus that causes erysipelas and one that is the toxic cause of a type of rheumatism, and yet their growth characters do not differ in respect of any feature, so far as has been ascertained, that would serve in all instances to distinguish the one from the other. It is to be hoped that in course of time distinctive features will be discovered, because it would be of great practical importance to be able to recognize in cultures the various pathogenic types.

Streptococcus pyogenes is generally present in small numbers in the normal mouth and pharynx. It is not found in the healthy nasal passages, intestine, or genito-urinary tract.

In association with acute and chronic inflammatory lesions of the respiratory and alimentary tracts, it is often present in enormous numbers. Scrapings from the inflamed surface show that the bacteria are lying among the tissues, and the practice of therapeutic immunization with autogenous vaccines has furnished ample proof that these invaders are exercising a pathogenic action. This proof consists in the occurrence of focal reactions and in the gradual repair of the lesions.

In the respiratory tract, *streptococcus pyogenes* is an important and fairly common cause of chronic nasal catarrh. It is very prone to invade the accessory sinuses. Many cases of chronic catarrh of the antrum and of the ethmoidal sinuses are dependent upon infection by this bacterium. In cases of chronic post-nasal catarrh *streptococcus pyogenes*

is rarely absent from the flora. It is liable to pass up the Eustachian tube, in which it may operate either as an acute or a chronic infective agent. This streptococcus is equally common as a bacterial factor in the causation of acute and chronic bronchitis.

In the alimentary tract, its first point of attack is the gums. In pyorrhœa alveolaris, and in the important condition of deep infection of the alveoli, which may occur without pyorrhœa, it is an almost constant pathogenic factor. In the latter condition, it is, as a rule, an anaerobe, and it is often associated in its action with an anaerobic strain of *micrococcus catarrhalis.*

Streptococcus pyogenes is the most frequent bacterial cause of acute and chronic tonsillitis and pharyngitis. It may attack the gastric mucosa and induce, or at least aggravate, acute and chronic catarrhal states. There is strong evidence in support of the conclusion that its localised action is the common cause of gastric and duodenal ulcer. The evidence rests upon the detection of the streptococcus in the stools, the occurrence of focal reactions in response to injection of autogenous vaccines and the gradual cessation of all symptoms under continued therapeutic immunization. I have observed three cases in which this succession of events has occurred. In my experience, no matter how severe may be infections by *streptococcus pyogenes* in the oral and naso-pharyngeal regions, if they are confined to these situations, this streptococcus does not appear in the stools. If, on the other hand, there is an infective lesion of the stomach or duodenum, or in the canal lower down, the streptococcus can be found in considerable numbers in the stools. I have observed sixteen cases in which it was present. In thirteen in which there was no suspicion of the occurrence of gastric or duodenal ulcers, the patients had other serious symptoms, differing, however, from case to case.

An officer in the army had suffered for ten years from recurrent attacks of pain in the region of the ascending colon, accompanied by rise of temperature, splitting headache, inability to digest any food, and intense malaise. These attacks generally lasted about three days. From time to time he had had attacks of severe eczema ; after each intestinal attack, there was some return of this trouble. In

1918, he suffered from neurasthenia, for which he was treated in a military hospital. No one ever suggested to him a bacteriological investigation of his intestinal flora. I found that his stools were loaded with two types of *streptococcus pyogenes*, one of them aerobic and the other anaerobic. Under therapeutic immunization with corresponding vaccines, the patient had focal and toxic reactions that exactly repeated his attacks in a mild form, and under continued immunization he made a good recovery. It is of interest to note that two other cases in the series suffered from eczema; in twelve, the streptococcus was associated with severe infection by an anaerobic diphtheroid bacillus and the patients were intensely neurasthenic.

In the skin, *streptococcus pyogenes* is often a factor in the causation of eczema. A sub-species with special pathogenic characters is the cause of erysipelas. Various strains are common infective agents in wounds, and they may lead to the development of a spreading cellulitis. *Streptococcus pyogenes* is probably the most frequent cause of puerperal uterine sepsis. It may attack the urethra. I have found it in four cases of chronic urethritis. Lastly, it may infect the blood-stream; it is, indeed, the most frequent cause of septicæmia.

Much that is, I believe, erroneous has been written, and is still generally taught, regarding the special pathogenic importance of hæmolytic strains of *streptococcus pyogenes*. Most strains are hæmolytic (in the sense of destroying hæmoglobin in a hæmoglobin agar medium, which does not coincide exactly with the hæmolytic test as generally applied in bacteriological work); some are intensely so; others are non-hæmolytic. Observation has shown, on the one hand, that some of the non-hæmolytic strains are extremely virulent, and on the other, that some of the intensely hæmolytic varieties do not exhibit any actions that would stamp them as having an unusually high degree of virulence. In short, hæmolytic power is not a criterion of virulence in this sub-group of streptococci.

It has been clearly established that several different varieties of *streptococcus pyogenes* are causes of simple rheumatism in its protean clinical manifestations. Sciatica, lumbago, muscular rheumatism, articular rheumatism, neuritis and iritis have all been shown by the method of focal

reaction and therapeutic immunization to have been due to the action of a streptococcus of this kind. In rheumatoid arthritis, which would appear to require other infective factors for its development, infection by *streptococcus pyogenes* generally complicates the clinical picture, adding some of the lesions of simple rheumatism to those of the graver malady.

On account of the belief of some that *micrococcus rheumaticus* is the pathogenic agent in acute rheumatism, and the rejection of this view of the causation of the disease by others (see Emery (32)), much uncertainty still exists regarding the bacterial origin of rheumatism. The question is specially discussed in the next chapter, but here I would state my belief, founded on my own observations, that the most common cause of simple rheumatism is a streptococcus of the *pyogenes* sub-group. There are, however, several distinct varieties—perhaps they should be called species—differing considerably in their characters, that are capable of causing rheumatism. One of the most common is one that appears as a short chained streptococcus, if special precautions are not taken. The long chains are shown only in a serum broth culture, and they readily break up when handled. If special care is taken in removing a portion of the mass from the broth, and in laying it on the slide, the fact will become apparent that the growth really takes place in long chains. This streptococcus is large, rounded, never bacillary, and it may occur either as an aerobe or as an anaerobe. It is generally the latter, and it will rarely be found capable of growth as both. The occurrence and detection of these anaerobic strains of *streptococcus pyogenes* in simple rheumatism is a matter of great practical importance. There are other quite different morphological types of *streptococcus pyogenes* that are also capable of causing rheumatism. There is especially one of very small size that grows in a broth as a feltwork; it also may be found either as an aerobe or an anaerobe. The streptococcus that has been regarded as *micrococcus rheumaticus* has, I believe, in some instances been *streptococcus fæcalis*, but probably more frequently it has been a *streptococcus pyogenes* of the variety that shows its long chained character only when special

precautions are taken. If anaerobic methods were systematically employed, it would probably be isolated much oftener. The gums, alveoli, tonsils, nasopharynx and (more rarely) the intestine are the most important seats of infection.

Persons suffering from chronic infections by *streptococcus pyogenes* generally feel chilly, and are given to sitting near the fire. They have cold extremities, and hence are prone to suffer from chilblains, and in some instances from arterial spasm and mild degrees of Raynaud's disease.

Therapeutic immunization against *streptococcus pyogenes* is generally highly successful, but it is of the utmost importance that a vaccine should be obtained corresponding to the particular type, or types, producing the malady. The initial dose is about 0·005 mg. In some cases 0·3 mg. may be reached towards the end of a course of treatment. Hypersensitiveness is rare, but does occasionally occur.

Streptococcus anginosus.—Like the other species of long-chained streptococcus that has just been considered, *streptoccoccus anginosus* is a very common and important pathogenic bacterium, with, however, a much less extensive range of action. It is found chiefly in association with acute and chronic inflammatory disorders of the gums, tonsils, nasopharynx and bronchi. Its occurrence is rare in the nose, intestine and genito-urinary tract, and it is never found in the skin. For the most part it simply causes local inflammatory lesions, thus often taking part in the production of pyorrhæa alveolaris, tonsillitis, chronic post-nasal catarrh and bronchitis. There is, however, clear evidence that in some subjects it is a very important cause of many forms of simple rheumatism. The dose is the same as that of *streptococcus pyogenes*—namely, from 0·005 mg. to 0·3 mg.

Streptococcus fæcalis.—This is a normal saprophyte of the intestine. On an agar surface, its colonies normally appear in proportion of about one to two hundred of those of the coliform bacillus. There is little evidence that it ever acts as a pathogenic agent. The view might be taken that it is capable of assuming a pathogenic action, and that, when it does so, it becomes hæmolytic. Many years of experience of both types incline me to regard them as separate species.

Streptococcus fæcalis is practically confined to the intestine.

Very occasionally, an apparently non-hæmolytic strain of a salicin-fermenting short streptococcus may be found in the nasopharynx, or adjacent mucous surfaces, but careful observation will generally show that it is really slightly hæmolytic.

Streptococcus fæcalis hæmolyticus.—This is a common pathogenic bacterium in the intestine, but is found also, not infrequently, in chronic lesions of the gums, nasopharynx, nasal passages, bronchi, urethra, bladder, and female genital tract. Its pathogenic action in the intestine is very commonly manifested in association with that of anaerobic diphtheroids. It seems to aggravate the neurasthenic disorders to which the latter give rise, and to cause especially states of slight mental confusion, inability to concentrate the thoughts and, in some persons, also headache, often of a severe character. These toxic effects may be manifested not only when it occurs as an intestinal infection, but also when it invades, as it frequently does, the nasopharynx and nasal passages. This streptococcus may be a cause of cystitis and it is common as a chronic urethral infection. It has also been found in the uterus in chronic inflammatory disorders of the organ.

A very interesting case of severe chronic nasal infection by this streptococcus came under my observation some years ago. The patient, a schoolmaster, suffered from an intense form of neurasthenia, headache and inability to concentrate his thoughts. Under therapeutic immunization he made a complete recovery.

Another case, that of a gentleman of middle age, suffered from a severe intestinal infection of this kind. He was unable to concentrate upon mental work, and suffered from a curious gripping sensation in his head. Under therapeutic immunization these symptoms disappeared. When he stopped the vaccines, his symptoms tended to return occasionally, and he continued their use for several years. From being an apparently hopeless neurasthenic, quite unfit for mental work, he became able to enter a university, and passed a series of professional examinations with credit.

The toxins of *streptococcus fæcalis hæmolyticus*, like those of *bacilli coli communis*, seem to exercise a pharmacological action of great importance, which must be considered quite separately from the immunizing response they are capable of eliciting in cases in which there is a corresponding infection.

Pneumococci.—The pneumococci form a large sub-group of pathogenic bacteria. Four different types have been identified as causes of acute pneumonia, specific and independent in their infective and immunizing powers (see references 34 and 35). There can be little doubt that a much larger number of independent strains occur as chronic infections. These facts give special importance to the use of autogenous pneumococcus vaccines. Polyvalent stock vaccines may be devoid of the special strain required, or may contain it in too small amount.

Pneumonia is not by any means the only acute disease that may be caused by pneumococci. An acute common cold, acute bronchitis, acute otitis media, acute arthritis in the course of an attack of pneumonia, puerperal infections of the uterus, acute meningitis, and abscess, may all be dependent upon a similar local infection. Chronic pneumococcus infections are far more common and important than is generally realized. They are a frequent cause of pyorrhœa alveolaris, stomatitis, tonsillitis, chronic rhinitis (often with extension to the accessory sinuses), post-nasal catarrh, otitis media, bronchitis, intestinal catarrh and conjunctivitis.

Chronic intestinal infections by pneumococci, apart from the infection in pernicious anæmia presently to be noticed, are of frequent occurrence and, at the present day, hardly recognized in medical practice. I have observed thirty-one cases of this kind in the course of the past five years. Most of the patients suffer from attacks of diarrhœa, but this symptom may be absent. All suffer from severe malaise, some are intensely neurasthenic, all are anæmic. Some strains of pneumococcus have a powerful neurotoxic action. My series of cases of intestinal infection included several of acute or chronic insanity in which there were good grounds for believing that the mental disturbances were dependent upon this infection.

A gentleman, aged sixty-three, had for over two years suffered from attacks of diarrhœa alternating with severe constipation. The intestinal flora was found to show about twenty streptococcus colonies to one coliform bacillus colony. On further investigation, about 70% of the streptococci were found to be pneumococci, and 30% *streptococcus fæcalis hæmolyticus*. Therapeutic immunization

was carried out against these two streptococci and against an aberrant type of *bacillus coli communis*. Under this treatment the patient made a rapid and complete recovery, and re-examination of the stools four months later revealed a normal flora.

A girl, two and a half years of age, had suffered for many weeks from attacks of diarrhœa, followed by severe constipation, anæmia and general malaise. Examination showed a pneumococcus infection of the intestinal tract. Under therapeutic immunization, the child made a complete recovery.

Numerous other cases could be cited to show the practical importance of intestinal pneumococcus infections and the readiness with which they generally yield to therapeutic immunization. The ordinary vaccine dose ranges from 0·005 mg. to 0·2 mg. Hypersensitiveness is common.

There are two types of pneumococcus infection which, on account of the special effects they produce, require separate detailed consideration.

Pneumococcus of Rheumatoid Arthritis.—That a special type of pneumococcus is the most common cause of rheumatoid arthritis is established by the study of a long series of cases. It infects most commonly the gums and nasopharynx, much more rarely the intestine. In the mouth and nasopharynx it may infect the tissues without causing any pain or discomfort. I have observed several cases in which it was found in extraordinary numbers in material scraped from livid patches on the gums or on other portions of the buccal mucosa.

This pneumococcus cannot as yet be distinguished from other pneumococci by any cultural or morphological characters. It is, as a rule, only slightly hæmolytic. The evidence in support of its being the cause of one type of rheumatoid arthritis rests upon the occurrence of the infection, the extraordinary hypersensitiveness of the patients to minute doses of a vaccine at the beginning of immunization, and the successful therapeutic results obtained in many cases.

The initial dose should be not more than 0·0001 mg. This, or a slightly higher dose, may cause exacerbation of the joint pain in from thirty-six to forty-eight hours. In such cases, if a dose of say 0·01 mg. is given, there will be a severe

aggravation of the malady with prolonged increase of pain. It is generally necessary to persevere with minute doses for several weeks. Hypersensitiveness ultimately disappears, and then the dose may be increased rapidly. It is sometimes possible to reach 0·05 mg.

Pneumococcus of Pernicious Anæmia.—That the commonest form of pernicious anæmia is due to an intestinal infection by a special type of pneumococcus, intensely hæmolytic in its action, I have known for many years. I have investigated thirteen cases bacteriologically, but have been able to carry out therapeutic immunization in only a few. The evidence bearing on the question is not, however, confined to the occurrence of the infection in recognized cases of pernicious anæmia.

The conclusion that this pneumococcus has a causal relationship to pernicious anæmia rests chiefly on the observation of the occurrence of the bacterium in the intestine in thirteen consecutive cases of the disease, and the intense sensitiveness of the patient to an autogenous vaccine. It is impossible to say that any case has been cured by therapeutic immunization. This fact has been regarded as proof that the pneumococcus infection has nothing to do with the disease. What the authorities recognize as pernicious anæmia is, however, really the terminal phase of a malady that has existed for many years before the blood changes have appeared. In this preliminary phase, the infection can be eradicated by therapeutic immunization. The subject is more fully considered in the next chapter under Pernicious Anæmia.

The dose of this pneumococcus, as of the preceding type, ranges from 0·0001 mg. to 0·05 mg.

Streptococcus salivarius.—This is a normal saprophyte of the mouth and is, I believe, never pathogenic.

Streptococcus salivarius, inulin fermenter.—There are grounds for believing that this is a pathogenic streptococcus of some importance. It may invade the tissues of the mouth and nasopharynx. Cases have been observed that have seemed clearly to show that it is one of the possible bacterial causes of rheumatic neuritis.

Streptococcus mucosus.—Like *streptococcus salivarius*, this

is a normal saprophyte of the mouth. I have never been able to obtain any evidence that seemed to point to its exercising a pathogenic action. Holman (*Journ. of Path. and Bact.*, April, 1915) attributes importance to it, but the streptococci he describes would, according to the criteria here adopted, be classified as varieties of pneumococci.

Streptococcus equinus. — This streptococcus, saprophytic in the intestine of the horse, occasionally causes acute catarrh of the respiratory tract in the human subject. I have observed one distinct case. The vaccine dosage may be regarded as the same as that of *streptococcus fæcalis hæmolyticus*.

3. Coli-Typhoid Bacilli

This is a large group, but consideration of some of its most important members hardly falls within the scope of a work on Therapeutic Immunization.

Bacillus coli communis. — This bacillus is a normal saprophyte of the human intestine. There are strong grounds for believing that its toxins, absorbed as they must normally be in considerable amount, exercise an important function in the human economy. It is certain that a vaccine prepared from the bacillus has a pharmacological action of much importance, and that it may be exploited especially in some forms of chronic ulceration with marked benefit to the patient. Its action seems to include a peculiar influence upon the vascular system. Varicose veins and varicose ulcers seem specially to benefit.

Whilst *bacillus coli communis* is a normal inhabitant of the colon, it is not found elsewhere about the body, except as a pathogenic agent. Thus its presence in the upper portion of the alimentary tract, in the respiratory system and in the genito-urinary tract indicates, with rare exceptions, that it is invading the tissues. Moreover, whilst in the colon it occurs normally as a harmless and even beneficial saprophyte, it nevertheless quite frequently in this organ assumes a pathogenic action, invading the tissues and producing local and general disturbances. One of the special dangers of such attack is the transference, by way of the communicating

lymphatics, of living bacilli to the urinary tract. There is clear evidence that these bacilli may pass through the urinary tract without attacking its walls. Very commonly, however, and especially in consequence of the patient happening to sustain a chill, the bacilli begin to invade and cystitis is set up, and, it may be, also pyelitis. There is great danger of this infection becoming a chronic one, if proper treatment is not carried out. An occasional, but rare, occurrence is the permanent infection of the urine without the production of cystitis. I have seen at least one remarkable case of this kind, in which the only inconvenience caused to the patient arose from the fact that his urine had always a disagreeable fishy odour.

Acute and chronic forms of cystitis due to infection of the walls of the bladder by *bacillus coli communis* are extremely common. In acute cases, therapeutic immunization is generally quickly effective ; in long-standing cases it is of great value, though the bacilli cannot be eradicated. The gall bladder is liable to a similar infection, but opportunities for making bacteriological examinations are very rare.

Bacillus coli communis occurs very occasionally as an infecting agent in the upper or lower respiratory tract. It rarely occurs in the oral cavity, but cases have been described in which it was found as a pathogenic agent in the stomach.

Aberrant types of *bacillus coli communis* are somewhat common, especially in the intestine, and there are good grounds for believing that they are in nearly every instance in which they occur exercising a pathogenic action. An aberrant type appearing in the intestinal flora must at least always be regarded with suspicion.

The dose of *bacillus coli communis* for therapeutic purposes ranges from 0·005 mg. to 0·1 mg.

Bacillus of Friedländer.—The bacillus of Friedländer may frequently be found as an element of the intestinal flora under conditions in which there is no reason to suppose that it is exercising a pathogenic action. That is to say, it may be a simple saprophyte. There are other cases, however, in which there can be no doubt that it is acting in the intestine as a pathogenic agent. It is one of the possible causes of chronic

and recurrent attacks of diarrhœa. Like *bacillus coli communis*, it may reach the urinary tract and cause cystitis.

This bacillus is also frequently to be found in the secretions from the upper or lower portion of the respiratory tract, and almost always as a pathogenic agent. It is one of the rarer causes of acute coryza. It may also occur as a chronic infection of the nose, and is apt to give rise to ozæna. The special type of Gram-negative bacillus described as the cause of ozæna is probably only an aberrant Friedländer. The nasopharynx may be the seat of a chronic invasion and both acute and chronic infections may occur in the bronchi. The bacillus of Friedländer is generally regarded as one of the causes of pneumonia, but it is certainly not a frequent one in this country. Pakes (*Brit. Med. Journ.*, 20th March 1897) attributes importance to it as an occasional cause of acute pharyngitis.

The therapeutic dose ranges from 0·005 mg. to 0·1 mg.

Bacillus typhosus, Bacillus paratyphosus A and B, Bacillus enteritidis.—In recent years, therapeutic immunization has been very successfully applied to infections by these bacilli. Allen (1 and 4) deals fully with the subject, and gives numerous literature references. There is much difference of opinion as to the suitable dosage. Some recommend an initial dose of only 2,000,000, and others one of 300,000,000. Allen states that experience seems to show that the best initial dose is one of 250,000,000 to 300,000,000, and that doses of this amount may be safely repeated at intervals of three to five days. Semple (23) has had very successful results. He recommends that treatment should be begun with a dose of 100,000,000 and that the amount should be increased to 300,000,000.

As is well known, protective inoculation is of great value as a preventive in persons who are likely to be exposed to infection by typhoid or paratyphoid bacilli. The pioneer work of Wright and Semple on the subject was carried out nearly twenty-five years ago (*Brit. Med. Journ.*, 30th Jan. 1897). The method generally employed is to give two injections at an interval of about a week, the first containing 500,000,000 bacilli and the second 1,000,000,000. The use of a mixed vaccine is now the rule, as it has been found necessary

to protect not only against *B. typhosus*, but also against *B. paratyphosus* A and B.

Some recent experiments of Besredka seem to show that protective immunization may be effected against typhoid, paratyphoid and dysentery bacilli by ingestion of the killed cultures, better than by subcutaneous injection of vaccines.

Bacillus dysenteriæ (Shiga, Flexner, etc.).—It was for a long time considered that therapeutic immunization was inapplicable to the treatment of bacillary dysentery, but it has now been successfully used by Forster and others (see Allen (1), p. 219). Protective inoculation by hypodermic injection does not appear to have been found practicable. There seems, however, to be a good prospect of a measure of artificial immunity being obtainable by methods of ingestion of the killed cultures, as suggested by Besredka (see *Brit. Med. Journ.*, 20th Aug. and 11th Sept. 1920).

Bacillus Morgan's No. 1.—Morgan found his bacillus in the stools of infants suffering from summer or epidemic diarrhœa, a malady of which it seems to be one of the most important of several possible infective causes.

Bacillus lactis aerogenes.—This bacillus has been found in the stools in cases of infantile diarrhœa of which it is believed to be a cause. In a case of chronic insanity I found it to be the only coliform bacillus apparently present in the intestine. Symptoms of intestinal disorder were absent, and there were no grounds for believing that the bacillus was acting as a pathogenic agent.

Bacillus acidi lactici.—This bacillus is non-pathogenic. It is one of the chief causes of the souring of milk.

Bacillus cloacæ.—This species occurs somewhat rarely as a pathogenic agent in any of the situations in which *bacillus coli communis* and the bacillus of Friedländer may be found. I have encountered it in only two cases. One was a case of pernicious anæmia with the usual intestinal pneumococcus infection. The aerobic flora consisted, in addition to the pneumococcus, of *bacillus proteus* and *bacillus cloacæ*. The other case was one of an ordinary type of post-nasal catarrh. The sputum contained *streptococcus pyogenes*, anaerobic *micrococcus catarrhalis* and this bacillus.

Bacillus fæcalis alcaligenes. — This bacillus has been

alleged to be an occasional cause of septicæmia. I have found it in nine cases of various kinds, in none of which did it certainly appear to be exercising an important pathogenic action. There were in every instance other infections sufficient to account for the morbid symptoms. In six of these cases the nasal or post-nasal passages were the seat of the infection, in two the intestine, and the bronchi in the remaining one.

Bacillus coli anaerogenes.—The pathogenic action of this species would indicate that it is merely an aberrant form of *bacillus coli communis*. Its occurrence is by no means frequent.

Bacillus oxytocus perniciosus.

Bacillus M'Conkey's No. 71.—These types were found by M'Conkey to be common in the intestine of man and of the lower animals. They are of little pathological interest.

4. Bacillus Proteus

The bacilli of this group are common bacteria of the soil, and of the alimentary tract of many lower animals. They are common agents of decomposition and putrefaction. That they frequently exercise a pathogenic action in man is clearly established.

Intestinal infection by virulent strains may produce severe intestinal catarrh with diarrhœa. The illness is generally recovered from spontaneously, but the bacillus may often be found for some weeks afterwards in small numbers in the stools. It may also become the agent of chronic intestinal infection. I have observed cases of this kind in which the complaint was diarrhœa unaccompanied by any severe constitutional disturbance. In some cases of toxic insanity, it has appeared to be of more serious import, probably aggravating a general toxæmia induced by other associated infections.

The intestinal tract is by no means the only possible seat of infection. *Bacillus proteus* is one of the micro-organisms that may contribute to the pathological process in pyorrhœa alveolaris ; it is one of the rarer causes of acute coryza. It may take part in a chronic post-nasal infection, and, when it

does so, it is extremely liable to pass up the Eustachian tube and to produce a severe form of otitis media. It is an occasional cause of acute or chronic cystitis.

I have notes of twenty-one cases in which *bacillus proteus* was obtained in the course of ordinary bacteriological examinations in the human subject. In nearly every one of these it was clearly exercising an important pathogenic action. It occurred in the intestine in eight cases, in the nasopharynx, or nose, in nine cases, in the bladder in two, and in the middle ear in two.

The therapeutic dose of the killed culture is similar to that of *bacillus coli communis*—namely, 0·05 mg. up to 0·1 mg.

5. Gram-Negative Diplococci

Micrococcus intracellularis (meningococcus).—It seems well established that this micrococcus is the cause of epidemic cerebro-spinal meningitis. Gordon and Andrewes (*Brit. Med. Journ.*, 23rd June 1917) have found that there are several epidemic strains which can be distinguished by agglutination tests. Other strains are unimportant. Treatment by therapeutic immunization appears to have no beneficial effect.

Gonococcus.—In its aerobic form, the gonococcus is the well-recognized cause of acute infective urethritis and vaginitis, as well as of a form of ophthalmia. The importance of chronic infections is still imperfectly recognized. As a chronic infective agent the gonococcus occurs, as a rule, as a bacterium that will grow only under anaerobic conditions. This fact has been brought to light through the systematic employment of anaerobic cultural methods in case investigations at the Laboratory of the Scottish Asylums. I have records of eighteen cases in which an anaerobic infection of this kind was found to be present in the genital tract. Eight of these were cases of a severe type of rheumatoid arthritis. Two were cases simply of chronic urethritis. The remaining eight occurred in women who suffered from various forms of disorder affecting the uterus, Fallopian tubes and ovaries, in each instance accompanied by severe reflex disturbances which differed considerably from case

to case, but which affected chiefly the gastro-intestinal tract. The existence of these anaerobic gonococcus infections was ascertained by the bacteriological examination either of a menstrual blood swab or of an intrauterine swab.

It is clear from the work of Allen (3), Brett (*Brit. Med. Journ.*, 28th *August* 1915), Lamb (*Brit. Med. Journ.*, 6th October 1917), and many others, that in acute cases larger doses than have generally been used are required. My own experience, chiefly in cases of chronic infection, entirely bears out this view. Initial doses of from 50,000,000 to 200,000,000 have been given. Brett recommends the latter initial dose, and gives a second of 1,000,000,000 after forty-eight hours.

Therapeutic immunization against acute infections may be begun with doses of 0·05 mg. As in all cases of acute infection the dose should be repeated at intervals of from one to three days. Probably a three-day interval is most suitable, as the amount of disturbance, especially general malaise and headache, produced is very considerable. If the doses are carefully regulated, the immunization may, however, be carried through without entailing undue suffering. It is of the utmost importance to keep increasing the dose, and to reach at least 1 mg. in the course of about ten injections.

Quite good results can be obtained in acute urethritis by means of ordinary vaccines, and the advocates of detoxicated vaccines have been rather unfair in their interested disparagement of them. The fact that some have given ordinary vaccines rashly and without sufficient knowledge of the strength of the emulsions they were using, or of the principles of dosage, can furnish no legitimate argument against their correct and scientific use. Whatever the advocates of the detoxicated vaccines may think of this truism, some of them have certainly acted in open contradiction of it.

It seems well established that there is some practical advantage in combining a *staphylococcus pyogenes* vaccine with the gonococcus one in the treatment of acute urethritis. The nature of the action is not clear, for *staphylococcus pyogenes* is by no means a constant or even common secondary infection. There must be some general action produced

by *staphylococcus pyogenes* toxins that is of value for the stimulation of the defences against the attack of many different bacteria.

Although gonococcal infection is known to be the chief cause of pyosalpinx, the pus is said to be sterile. It is probable that, if blood anaerobic methods of culture were employed, growth would be obtained.

Immunization against anaerobic gonococcus infections must be carried out with corresponding vaccines. The dose is from 0·05 mg. to 1 mg., and the intervals should be not less than one week. Several of the cases I have treated were at first intensely hypersensitive to the toxin.

Micrococcus Melitensis.—This micrococcus is the cause of Malta fever. Therapeutic immunization has been successfully applied both in acute and chronic forms of the disease. The initial dose appears to be from about 0·02 mg. to 0·04 mg. References to several papers on the subject are given by Allen (1), p. 290.

Micrococcus catarrhalis.—That numerous strains of diplococci having the special characters that distinguish *micrococcus catarrhalis* are causes of acute, chronic and recurrent catarrh of the respiratory tract is now clearly established. This diplococcus is the most frequent cause of common colds. Epidemic strains are highly infectious. One of them will often sweep rapidly over the population of a town or country district, from person to person, picking out those who are susceptible, and taking advantage of any temporary weakening of the bodily defences entailed by chill and fatigue in order to launch its attack.

Micrococcus catarrhalis is also one of the frequent and important causes of chronic and recurrent catarrh of the respiratory tract. It is, however, rarely to be found as the exclusive infective agent in such cases. *Streptococcus pyogenes, streptococcus anginosus* and the bacillus of influenza are probably its most usual associates. It may invade any of the accessory sinuses of the nose and the Eustachian tube. It is, further, often an important element in the infective causation of pyorrhœa alveolaris. It may be one of the invading bacteria in chronic tonsillitis.

Micrococcus catarrhalis may be found in a form that grows

only under anaerobic conditions. This type is of great pathological importance and probably very many failures of therapeutic immunization have been due to its presence not having been detected. It occurs especially in chronic post-nasal catarrhs, chronic bronchitis and pyorrhœa alveolaris. It invades the alveoli along with anaerobic strains of *streptococcus pyogenes*, and the two are so often found together that they would almost appear to have some symbiotic relationship to each other. Patients suffering from deep alveolar infection of this kind are generally intensely rheumatic, but I have been unable to discover any evidence that the anaerobic diplococcus has a pathogenic action similar to that of the streptococcus. It certainly occurs in other situations quite commonly in persons who have not a trace of rheumatism.

The bacillary type of *micrococcus catarrhalis* may be observed with some frequency in the respiratory tract and mouth. It may be the agent of an acute, or of a chronic infection. I found it in great numbers in the nasal passages in one case of acute coryza.

The dose of *micrococcus catarrhalis* ranges from 0·05 mg. to 0·5 mg.

Micrococcus pseudo-catarrhalis.—The many varieties of pseudo-catarrhalis diplococci are commonly regarded as non-pathogenic bacteria. While, for the most part, it is probably true that they are mere saprophytes, there are grounds for believing that among them there are types that may assume a pathogenic action. When they are present in large numbers in a case of chronic catarrh, it is often worth while including them among the species against which therapeutic immunization is to be directed. On account of their stimulating action on the growth of *bacillus influenzæ* and *bacillus pertussis*, they should, when present, always be included in the immunization in cases of chronic infection by these bacilli. I have observed several cases in which neglect of this rule was attended by failure to suppress the bacillary infection by autogenous vaccines, and in which success was almost immediate when a pseudo-catarrhalis vaccine was added.

The range of dose is the same as that of *micrococcus catarrhalis*.

6. The Bacillus of Diphtheria

Acute diphtheria is treated successfully by antitoxin. It is in chronic forms of the infection, which are commoner than is generally supposed, that therapeutic immunization has its important application.

A combination of vaccine and antitoxin treatment of acute diphtheria has, however, recently been employed, apparently with much success, by F. M. Wood (see *Lancet*, 28th Feb. 1920, p. 510). He gives doses of from 300,000,000 to 500,000,000 bacilli.

I have observed three cases of chronic infection by this bacillus. One case was that of a patient who suffered from a severe form of chronic bronchitis, and who on one occasion ejected a membranous cast of the bronchi. The second was that of an army officer who suffered from severe nasal catarrh and repeated epistaxis, and who was known to have had diphtheria about two years before ; and the third was that of a man who had chronic nasal and post-nasal catarrh and symptoms of neuritis, and whose nasopharynx was found to be infected by this bacillus along with other pathogenic bacteria.

The treatment of carriers by therapeutic immunization is now well recognized as being effective. The dose of the vaccine ranges from 0·05 mg. to 0·5 mg.

7. Diphtheroid Bacilli

There are very few authorities in this country, or on the Continent, who, at the present day, attach much importance to bacilli of the diphtheroid group as pathogenic agents. The text-books generally refer to them merely because they have to be distinguished from the bacillus of diphtheria. The fact that *bacillus acnes* is a member of the group is rarely recognized. Regarding other anaerobic species, there is complete silence. It must be said, however, that much more attention has been paid to the subject in America. In this country, Llewellyn Heath (*Journal of Vaccine Therapy*, 1913, p. 219) has recorded the observation of the presence of diphtheroid bacilli in various acute and chronic lesions.

Morriston Davies and George Hall (*Brit. Med. Journ.*, 11th Jan. 1908) have described a bacillus in cases of neuropathic keratitis that is clearly a diphtheroid.

The results of my own observations on the subject, extending over more than twenty years, lead me to regard the diphtheroid bacillus group as one of the most important in human pathology. In 1914 I collected the evidence I had obtained up to that time, and prepared a paper entitled *The Pathological Importance of Bacilli of the Diphtheroid Group* for one of the sections of the Annual Meeting of the Royal Institute of Public Health, held in Edinburgh in July. The shadow of the impending European catastrophe darkened that meeting, and the section held only a single sitting. My paper was therefore never read. I offered it shortly afterwards to a leading medical journal, but the editor returned it as not being of sufficient interest for publication. The full import of this verdict cannot be appreciated without a knowledge of some of the things that this editor, about the same time, deemed of sufficient interest to occupy many times the space that my paper would have required. I print this paper here and ask the reader to regard it simply as a record of the facts that had been ascertained up to the time at which it was written:

By the diphtheroid group of bacilli I mean one that comprises all the small, rod-shaped, Gram-fast bacteria that exhibit metachromatic granules (at least at some stage of their growth), with the exception of the bacillus of acute diphtheria. It is, in my opinion, of no practical value to include, as some do, bacilli that never contain metachromatic granules, such as Hofmann's bacillus (as commonly described) and the *bacillus septus*. A suitable test for the presence of metachromatic granules is staining with Neisser's methylene blue. In preparations by this method metachromatic granules have a purple tint which contrasts sharply with the blue colour of the rest of the bacillary body. The group, even as thus limited, probably contains numerous separate species, but, so far as I am aware, no satisfactory criteria for their differentiation have yet been discovered. In the present position of knowledge, the bacillus of acute diphtheria, or the Klebs-Löffler bacillus, is distinguished, not by any special morphological characters, but by its virulence to guinea-pigs, and by its power to produce acid when grown in a dextrin test broth. That these distinctions are absolute is not altogether certain; any observations that tend to throw doubt upon their validity are certainly rare.

The bacilli of the diphtheroid group as thus narrowly, and also as generally more widely, defined, are at present regarded by nearly every authority upon bacteriology as devoid of pathogenic power. The stock arguments against their importance are especially two, which, though mutually contradictory, have sometimes been canvassed simultaneously by the same writer. They are, first, that diphtheroid bacilli are "ubiquitous," and second, that they cannot be found where persons who have attributed importance to them allege they are always present. Now the first of these arguments, stripped of the hyperbolic language in which it has been expressed by an eminent authority, is of no force; it is equally applicable to other groups of bacteria the pathological importance of which is established, such as those of the coli-typhoid bacilli, the Gram-negative diplococci, the streptococci and the staphylococci. In each of these four groups there are harmless common saprophytes, as well as important pathogenic bacteria, and many, indeed probably all, of the pathogenic forms may at times have a purely saprophytic existence at some mucous surface of the human body. The case is exactly the same in regard to the bacilli of the diphtheroid group. It is invasion of the tissues, not mere presence at a surface that stamps a micro-organism a pathogenic agent. The negative observations that some investigators have been at so much trouble to record are of a kind that it is very easy to obtain, and these workers have done themselves little credit by their failure to observe prominent facts the reality and importance of which it would now be futile to deny. Many bacteriologists have allowed themselves to be gravely misled in this matter by assuming that all diphtheroid bacilli can be grown upon an agar or glucose agar medium. Some members of the group are among the most fastidious bacteria that can be encountered in bacteriological work. I quite commonly meet with strains that will not grow upon a sugar agar (lactose agar is the kind I use). As I pointed out some years ago, what the more delicate strains in the group require for their growth is free hæmoglobin. Solidified serum without hæmoglobin, and even human blood agar are insufficient and, indeed, present little advantage over a sugar agar. I use a sloped hæmoglobin agar, prepared by adding to ordinary nutrient agar (sterilized in the autoclave and allowed to cool to about 50° C.) from 5% to 10% of hæmoglobin serum, obtained by freezing and then thawing sheep's blood secured with precautions against external contamination.

I maintain that various species included in the diphtheroid group of bacilli are important pathogenic organisms in the human subject, and I shall now briefly present some of the evidence I have collected that seems to me to warrant this conclusion.

The Occurrence of Diphtheroid Bacilli in the Human Subject

Diphtheroiduria.—It has been proved that normal urine, obtained with due precautions against contamination from the exterior, s

sterile. I have records of sixteen cases of various forms of infection of the urinary tract in which diphtheroid bacilli could not be grown from the urine, so that these organisms are not constant accompaniments of other infections of this tract. One of the most striking facts that has come to light is the great frequency of diphtheroiduria in cases of well-marked neurasthenia. I have found very abundant diphtheroid bacilli to be present in the urine in twenty cases of this nature, which were not accompanied by the more grave maladies of which neurasthenia is sometimes the prelude. It would indeed appear to be the rule that severe neurasthenia is associated with diphtheroiduria. The number of colonies is generally not proportionate to the number of bacilli that may be seen in a stained film of the centrifuge deposit, in which, with suitable staining, it may be observed that most of the micro-organisms are in process of disintegration. I have also found severe degrees of diphtheroiduria in cases of exophthalmic goitre (4), myelitis (3), in several of mental disorder, marked especially by confusional symptoms, in which a diagnosis of general paralysis or of dementia præcox could be excluded, and in a series of fourteen consecutive cases of dementia præcox at an early stage of the disease. The number of cases of tabes dorsalis in which I have made a bacteriological examination of the urine extends to considerably over fifty. In this malady there is, in my experience, always a well-marked diphtheroid infection of the urethra, except in some very advanced cases in which it is displaced by other infections, such as those by a colon bacillus and the *diplococcus crassus*. In cases of general paralysis there is also, almost constantly, a well-marked diphtheroiduria, but it may be of the neurasthenic type—that is to say, the bacilli are in process of disintegration, having, in fact, been absorbed from an infective focus and excreted from the blood by way of the kidneys.

Infection of the walls of the urethra and bladder by diphtheroid bacilli may occur from without, being in this case generally secondary to a gonococcus infection, or from within, sometimes independently of any previous acute infection, in consequence of excretion of the living micro-organisms by way of the kidneys. In women such diphtheroid infections are apt to extend to the genital tract.

Diphtheroid Bacilli in the Nasal Passages.—It has been stated by some authorities that diphtheroid bacilli with metachromatic granules do not play any part in infections of the nasal mucosa. In my experience such micro-organisms are very commonly present in large numbers in cases of acute and chronic rhinitis. They are sometimes present alone, but more commonly they are accompanied by other bacteria, such as *micrococcus catarrhalis*, the pneumococcus, the bacillus of Friedländer, and *bacillus influenzæ*. In cases of general paralysis the nasal discharge would appear to be constantly loaded with diphtheroid bacilli. I have records of over fifty examinations in which positive results were obtained. Since I began to use hæmoglobin agar some years ago, every case of general paralysis

examined has yielded abundant diphtheroid growths. The earlier the case, the more striking is the demonstration that may be given of the severity of this local infection. In advanced cases, secondary infections often obscure the diphtheroid one. It may here be added that in many cases of mild chronic conjunctivitis a bacillus with metachromatic granules (which most authorities would classify as a xerosis bacillus, although metachromatic granules are commonly denied to this organism) can be obtained from the conjunctiva in pure culture, or along with other bacteria.

Diphtheroid Bacilli in the Alimentary Tract.—Diphtheroid bacilli are ordinary saprophytes of the mouth, in common with various species of Gram-negative diplococci, streptococci, staphylococci, spirochætes, etc. It is well known that scrapings and cultures from the inflamed gums in cases of pyorrhœa alveolaris almost always contain abundant diphtheroid bacilli. It is not, however, so generally recognized that these bacilli may occur also in abundance in the stools. In cultures from such material they are obscured by the vigorous growth of coliform organisms, and are consequently somewhat difficult to isolate. In cases of intestinal disorder, especially in neurasthenics, they may commonly be observed in direct films.

Cultures from chronic inflammatory lesions of the skin often yield diphtheroid bacilli along with other bacteria. Syphilitic ulcers are specially liable to be the seat of such infection.

In the foregoing summary I have omitted reference to the demonstration of diphtheroid bacilli in the blood and cerebro-spinal fluid in various morbid conditions, because these observations have an additional value as evidence of pathogenic action. They are included in the next section.

Evidence of Pathogenic Action of Diphtheroid Bacilli

The evidence I have to give in support of the view that these bacilli frequently exert a pathogenic action is just of the kind that satisfies us in regard to other micro-organisms—namely, that drawn from animal experiments, from evidence of invasion in association with inflammatory lesions in the human subject, focal reactions to vaccines and the therapeutic action of vaccines.

Experimental Evidence.—I have tested the virulence of these bacilli by hypodermic injection of 1 c.c. of a broth culture in guinea-pigs and mice. It will suffice if I give the results obtained with strains isolated from cases of general paralysis and tabes dorsalis, although diphtheroid bacilli from other sources have also, in several instances, proved virulent to mice.

Out of 61 strains isolated from 41 cases of general paralysis there were 18 that showed virulence to mice, or about 26%. Sixteen out of 135 mice injected died within forty-eight hours, and 8 others within a period of six weeks. In several instances a particular strain was fatal within forty-eight hours to both of two mice injected. Out of 44 strains

from 31 cases of tabes dorsalis, 8 showed virulence to mice, or 18%. The two series put together show 23% of the strains to have been virulent to mice. It will be remembered that the Klebs-Löffler bacillus, although virulent to guinea-pigs, is innocuous to mice.

Only one guinea-pig died in each of the two series of experiments. Altogether sixty-one strains were tested, one animal being used for each.

The virulence has also been tested by other experimental methods in rats and rabbits. It has been shown that rats fed with some strains become demented and paretic, and may die paralysed after a few weeks or months. This observation has been confirmed by Flashman in Australia. Some strains of diphtheroids produce no such effects upon rats. A number of rabbits have been injected intraspinally with living cultures. Experiments of this nature have, however, a somewhat doubtful value, because any micro-organism so introduced must set up local reactive changes. Very severe lesions were caused by these intra-spinal injections of diphtheroid bacilli, isolated from cases of general paralysis and tabes, periarteritis of the vessels of spinal cord, accompanied by abundant plasma cell development, being a well-marked feature. A striking fact brought out was that cultures made from the cerebro-spinal fluid a few days after the injection of the living bacilli would not yield a growth, and yet, in several of the rabbits, the lesion progressed and the animals died completely paralysed in their hind limbs.

Two sheep were injected in the nasal mucosa with a culture of a diphtheroid bacillus isolated from the nose of a general paralytic. One of the animals had previously been immunized by hypodermic injection of bacilli of the same kind. The other had not been immunized. The immunized sheep was not affected in any way; the other developed symptoms of acute delirium and died on the twelfth day after the injection.

Injection of the genital tract in rabbits has frequently been followed by the development of dullness and stupidity in the animal, and also in some instances by paresis of the hind limbs.

Evidence of Invasion in the Human Subject

Evidence that diphtheroid bacilli may exert a pathogenic action upon the human subject is afforded by the demonstration of the fact that they frequently invade the living tissues. These bacteria do not grow at room temperature, and therefore their presence in the tissues after death is of value as evidence of infection. Many of the observations that I have to record have been made, however, upon material obtained during life.

Histological examination of the urethra in cases of general paralysis has shown that the bacilli lodge in the mucous glands, and from these invade the submucous tissues. An extensive histological examina-

tion of the nasal mucosa from cases of the same disease has proved that deep invasion of the tissues by diphtheroid bacilli is of almost constant occurrence and that it is associated with well-marked chronic inflammatory changes. The bacilli have been traced along the lymphatics to the base of the brain.

Scrapings taken from inflamed gums in fourteen cases of general paralysis during life have all shown diphtheroid bacilli among the tissues. In a case of dementia præcox with severe pyorrhœa alveolaris I made a bacteriological examination of material from an ulcerating gum. The usual very varied flora was obtained; diphtheroid bacilli were present, but did not appear to be specially abundant. A piece of the gum was excised from the same place, and examination of stained sections revealed the remarkable fact that the only bacterium that could be seen to be invading was a diphtheroid bacillus with metachromatic granules.

In several cases of general paralysis extensive invasion by diphtheroid bacilli has been found in sections of the bronchial and intestinal tissues. In three cases of endometritis, the patient being in each instance the wife of a general paralytic, I was afforded an opportunity, by the gynæcologist who was called in, of examining scrapings of the uterine mucosa. In all these cases the results were similar. Pure and abundant growths of diphtheroid bacilli, similar to those obtained from the husband, developed in the culture tubes, and histological examination showed invasion of the tissues by the same micro-organisms. In the absence of other morbid factors, it seems reasonable to conclude that these invading bacilli were the cause of the chronic inflammatory process. In one of the cases the bacilli were very virulent to mice.

A special observation made in a case of general paralysis also illustrates the fact of local invasion and pathogenic action. This patient from time to time passed prostatic casts. Some of these were taken from the urine and carefully washed in normal salt solution. Direct examination showed that they consisted mainly of polymorphs, among which diphtheroid bacilli were present in large numbers. Cultures yielded pure and abundant growths of an organism of the same kind.

In conjunction with colleagues, I have made cultures from the blood in twenty-three cases of general paralysis. In seven of these a diphtheroid bacillus was obtained. I have made cultures from the cerebro-spinal fluid in fifty cases and have obtained a growth of diphtheroid bacilli in six; in fifteen of them diphtheroid bacilli were demonstrable in the centrifuge deposit from the fluid. In America Dr J. D. O'Brien has obtained similar growths in a much larger proportion of cases.

Occurrence of Focal Reactions to Vaccines

Evidence that these bacilli may exert a pathogenic action is also afforded by the occurrence of focal reactions to minute doses of

autogenous vaccines. I have now observed such focal reactions in numerous cases, and they have occurred also in the experience of others who have used such vaccines. For example, a lady who suffered from chronic nasal catarrh and slight chronic conjunctivitis was found to have a severe infection of the nasal mucosa by a diphtheroid bacillus and the bacillus of Friedländer. An initial dose of 0·04 mg. of an autogenous diphtheroid vaccine produced next day not only a copious discharge from the nose, but intense congestion of the conjunctiva. Intestinal, urethral and uterine focal reactions have also been observed in cases of corresponding local infection by diphtheroid organisms. In one case of urethral infection a condition of hypersensitiveness has been observed, the focal reaction to the most minute dose being so severe that treatment had to be abandoned.

Effects of Treatment with Autogenous Vaccines

Evidence of pathogenic action of diphtheroid bacilli has also been afforded by the results of autogenous vaccine treatment in various types of cases. In my experience, neurasthenics often benefit greatly by a course of treatment with autogenous vaccines. Under similar treatment, early cases of tabes improve to a marked extent. Cases of general paralysis, on the other hand, have shown no permanent improvement.

Conclusions

Invasion of the tissues by diphtheroid bacilli is of very common occurrence. The infection is generally chronic, often persisting for years, and, indeed, when once established, tending, like coliform infections of the urinary tract, to be permanent. The chief seat of invasion is the mucosa of the alimentary tract, and more especially of the mouth, the lower end of the ileum and the colon. In sections, the bacilli can be seen lying among the epithelial cells and sometimes also in the neighbouring lymphatic channels. From these situations they tend to be carried to the blood-stream. Those that escape destruction are excreted by way of the kidneys, in the tubules of which they have been demonstrated. In the urine they may occur exclusively as disintegrating and dead bacilli, though, as a rule, a growth can be obtained.

This process of slow absorption from infective foci must be attended by special toxic actions, varying probably to some extent with the particular species of diphtheroid bacillus. Experimental and clinical evidence shows that these actions are chiefly neurotoxic in character. Chronic infection by diphtheroid bacilli occurs especially in diseases of the nervous system, including certain types of neurasthenia, acute toxic insanities, general paralysis, tabes dorsalis and dementia præcox. In general paralysis and tabes it is perhaps always associated with syphilis; in dementia præcox the special accompanying

condition would appear to be an inherited greater liability to the fixation of the toxins by the cortical nerve cells.

With regard to general paralysis a few special words are necessary. There is at the present moment no actual proof that infection of the brain by the *spirochæta pallida* is a complete explanation of all the characteristic phenomena of this disease. It would be easy to show how extremely weak are some of the links in the chain of evidence that at present satisfies nearly every authority on the subject. I still maintain, as I have done for many years, that the toxic actions in general paralysis are essentially bacterial. The conclusion that agrees most fully with the bacteriological evidence of the kind of which I have given a summary in this paper is that the cerebral vessels have been damaged by a local syphilitic infection in such a way that they have been rendered in places permeable by bacteria circulating in the blood. The general paralytic always has well-marked infective foci in his alimentary tract, often also in other situations. These foci are commonly the seat of mixed infections, but investigation has shown that the only micro-organism that reaches the blood-stream in large numbers may be a diphtheroid bacillus. The general paralytic, in common with some neurasthenics and the sufferer from dementia præcox, is excreting diphtheroid bacilli in the urine, but he differs from them in respect of the circumstance that his cerebral vessels are permeable, so that the bacteria are poured into the cerebral lymph spaces, as well as into the urinary tract.

If these views are well founded, as I believe them to be, then chronic infection by diphtheroid bacilli, or diphtheroidosis, as it might conveniently be termed, is a very common, widespread and important cause of illness.

In the course of the seven years that have passed since the foregoing paper was written I have made many additional observations that have served to strengthen the evidence in support of the conclusion that bacilli of the diphtheroid group are of great practical importance as causes of disease, and that their action is chiefly neurotoxic. The systematic employment of anaerobic methods in case investigations has served to add one outstanding fact—namely, that many of the important species in this group are anaerobes, which occur chiefly, but not exclusively, in the intestine. In normal conditions, and in most cases of acute and chronic bacterial infection of the intestinal tract, anaerobic diphtheroid bacilli do not appear in the culture tubes. In all cases in which they are present in large numbers the patient is suffering from nervous symptoms, always of a neurasthenic

character, but frequently with more grave disturbances superadded. Thus, in all of sixty-three cases in which I have found anaerobic diphtheroid bacilli to be a prominent feature of the intestinal flora, the patients had well-marked neurasthenia; twelve of them suffered also from exophthalmic goitre and several presented distinct mental disturbances. It must not, however, be thought that an intestinal anaerobic diphtheroid bacillus infection occurs in every case of neurasthenia; there are several important bacterial causes of this malady, and there are cases in which the important pathogenic factors are not bacterial. Anaerobic diphtheroid bacillus infections also occur, though less commonly, in the nasopharynx, gums, bronchi, bladder, urethra, prostate gland and uterus, and it is important to know that invasion from any of these situations may produce severe neurasthenic symptoms. Specially severe types of neurasthenia arise from chronic infection of this kind involving the prostate gland, or the uterus. Aerobic types commonly infect any of the situations named and also the nasal passages, conjunctiva, skin and chronic ulcers and sinuses. Some of these aerobic forms are intensely neurotoxic, others seem devoid of this character. From whatever situation aerobic or anaerobic diphtheroid bacilli invade, they are liable to reach the blood stream, from which they are excreted by way of the kidneys and urinary tract. In cases of acute toxic insanity and general paralysis of the insane, they have frequently been cultivated from the blood. In harmony with the neurotoxic character of the diphtheroid group is the fact of the great prominence of aerobic and anaerobic diphtheroid bacillus infections in cases of insanity. In the acute insanities and in manic depressive cases, anaerobic intestinal infections are very common. In dementia præcox, there is very often a severe nasal infection by aerobic diphtheroid bacilli, and the stools and the urine are frequently loaded with aerobic or anaerobic forms. Diphtheroid bacilli without doubt commonly occur as saprophytes at mucous surfaces, more especially of the genital tract, but this fact need not detract from their importance as pathogenic agents in other instances. The evidence derived from focal reactions, from intense hypersensitiveness to the auto-

genous vaccine in some cases, and from the success of therapeutic immunization in relieving distressing symptoms, has clearly established the reality of the pathogenic action of these bacteria.

A fact worth noting is the apparently constant prominence of aerobic or anaerobic diphtheroid bacilli in the intestinal tract of persons suffering from psoriasis.

The therapeutic dose of bacilli of this group varies greatly in accordance with the species. Some patients show intense hypersensitiveness to the neurotoxic strains, and doses of no more than 0·001 mg. to 0·005 mg. may be tolerated. In cases of neurasthenia with anaerobic intestinal diphtheroid bacillus infection, an initial dose of from 0·01 mg. to 0·05 mg. is generally suitable, and a maximum dose of about 0·4 mg. may usually be reached in the course of ten or twelve injections.

8. Influenza Bacillus

Two members of this group, *bacillus influenzæ* and *bacillus pertussis*, are of great moment as causes of acute and chronic illness; the third, the Koch-Weeks bacillus, is of minor importance.

Bacillus influenzæ.—This bacillus, discovered by Pfeiffer, has long been generally accepted as the infective cause of influenza. Attempts have recently been made, however, to discredit this belief, and to show that the true cause of the disease is another bacterium, which is a filter passer. This view is regarded by many as being based upon unsatisfactory evidence. Its advocates are, I think, much to blame for the lack of scientific impartiality they have shown in ignoring the weighty claims of the *bacillus influenzæ* to be regarded as the real pathogenic agent.

That *bacillus influenzæ* is the specific cause of epidemic influenza seems to me to be established on the following grounds:—(1) The bacillus can be found in abundance in most cases of the acute disease; in the few in which it cannot be obtained, the failure may easily be accounted for by the special difficulty there is in growing the bacillus, and by the fact that, in the early stages of the disease, it occurs in the tissues and is not thrown off in the secretions in any

large numbers. Allowance must also be made for the fact that during influenza epidemics febrile attacks due to other causes are often mistaken for influenza. I have investigated many cases of this kind, obtaining negative results as regards the presence of the bacillus of influenza, but have generally found evidence of other acute infections that accounted for the symptoms. (2) In cases of influenza, confirmed by bacteriological investigation, therapeutic immunization, properly carried out, has been found to cut short the malady. (3) It has been satisfactorily established that preventive inoculation against the bacillus of influenza protects from the disease. (4) A certain proportion of persons who suffer from chronic infection by the bacillus of influenza have typical attacks of influenza at intervals of a few weeks. I have treated several cases of this kind by therapeutic immunization. In all of them the attacks immediately ceased, and the patients were soon restored to health. If the *bacillus influenzæ* was not the cause of the attacks of influenza in these cases, therapeutic immunization against it could not have arrested the patient's malady. (5) Even in normal persons, but especially in those who suffer from chronic infection of the respiratory tract by *bacillus influenzæ*, an overdose of the corresponding vaccine produces symptoms that are exactly those of acute influenza. (6) Persons who suffer from chronic infection by the bacillus of influenza, whilst they continue to have their own characteristic symptoms, are not subject to acute infection during an epidemic of influenza. If some other bacterium is the cause of epidemic influenza, there is no reason why these persons should be immune to its attack.

Important experimental evidence in support of the conclusion that the bacillus is the cause of influenza has recently been obtained in America by F. G. Blake and R. L. Cecil, who have successfully produced the disease in monkeys by means of strains of *bacillus influenzæ*, the virulence of which had been raised by passage through mice and monkeys (see *Brit. Med. Journ.*, 15th Jan. 1921, p. 94).

Most people regard the bacillus of influenza as a bacterium that visits us only occasionally, causing more or less severe epidemics. As a matter of fact, it is always with us; it

occurs quite commonly as the agent of a chronic infection. There are evidently numerous more or less distinct strains, differing considerably in virulence and in shades of pathogenic action. The great epidemics of influenza that from time to time sweep over large areas are due to a variant of special virulence that has secured a firm footing. Each epidemic thus started has its special features. After some months, the epidemic strain either dies out, or loses its virulence, and ceases to be capable of inducing an epidemic. There are good grounds for the belief that, after an epidemic has passed, the number of cases of chronic infection by the bacillus remains greatly increased for many months.

In acute influenza, the bacillus infects chiefly the respiratory tract. It is very prone to attack the accessory sinuses of the nose. Pneumonia is one of the commoner, and acute meningitis one of the rarer, complications that may occur. As a rule, there are important associated infections, among the most important of which are those by pneumococci, *streptococcus pyogencs* and *micrococcus catarrhalis*. It is well established that diplococci of the catarrhalis group stimulate the growth of the bacillus in culture, and there are grounds for believing that they do so also in the respiratory tract. Indeed, epidemics of infective cold due to *micrococcus catarrhalis* and of true influenza seem often to occur simultaneously, the action of the micrococcus preparing the way for the attack of the bacillus. It must not, however, be thought that such infection by *micrococcus catarrhalis* is an essential preliminary to attack by the influenza bacillus.

The special difficulty that there is in growing the bacillus of influenza is probably the chief reason for which its occurrence as a chronic infection is so rarely observed and so imperfectly understood. As a matter of fact, chronic infections are very common, and they are of the utmost practical importance. They cause much suffering, and the only known remedy is therapeutic immunization, which acts rapidly and with uniform success. In the course of the past five years I have observed 107 cases. A brief summary of the main facts brought out by the study of 100 consecutive cases may be of interest here.

The symptoms of chronic infection by the bacillus of

influenza are not altogether characteristic; other chronic infections of the respiratory tract, especially in combination, may produce very similar effects. Chronic bronchitis with periodic exacerbations occurred in 46; asthma complicated this trouble in 12. There were symptoms of post-nasal catarrh in 44. Rheumatism was a well-marked symptom in 14. Severe neurasthenic symptoms occurred in 5. The Eustachian tubes were involved in 4 cases, and the patients complained of deafness. Acute mental disorders occurred in 6. There was mental depression in 3 others. Insomnia was a troublesome symptom in 2 cases. A toxic action was exhibited upon the heart in 3 cases, resulting in extraordinary hypersensitiveness of the organ to a vaccine. The bacillus was found in various situations as follows:— bronchi, 44; nasopharynx, 51; gums, 13; fauces, 3; nose, 3; accessory sinuses of the nose, 2; urethra, 1.

These cases are always complicated by associated chronic infections of importance. The most common accompanying pathogenic bacteria are *micrococcus catarrhalis, streptococcus pyogenes,* pneumococci and diphtheroid bacilli. A fact little known is that in a large proportion of cases of pyorrhœa alveolaris the gums are infected by this bacillus. I have found it in thirteen cases out of fifty-one. One of the rarer sites for a chronic infection by the influenza bacillus is the urethra. I have seen only a single case, but Allen has observed several.

The following are notes of some typical cases:—

No. 38 was that of a student who had suffered since early childhood from frequently recurring attacks of severe nasal and post-nasal catarrh. Each attack began with drowsiness; this was quickly followed by aching throughout the body, rise of temperature, nasal catarrh and complete prostration. The illness would generally last for three or four days, after which he quickly recovered, but only to be prostrated again after a few weeks by a similar attack. A post-nasal swab yielded profuse growths of *bacillus influenzæ, streptococcus anginosus, streptococcus fæcalis hæmolyticus, micrococcus catarrhalis* and diphtheroid bacilli. Under corresponding therapeutic immunization the patient made a complete recovery.

No. 48 was the case of a young lady who for several years had suffered from attacks very similar to those experienced by the preceding patient, but involving also the lower respiratory tract. The whole respiratory tract was infected by the bacillus of influenza, and

there were associated infections by *streptococcus anginosus*, pneumococci, *staphylococcus pyogenes aureus* and diphtheroid bacilli. Under therapeutic immunization the patient made a rapid and complete recovery and after several years remains well.

No. 36 was the case of a lady whose efficiency as the matron of a hospital was seriously impaired by frequent prostrating attacks of catarrh that were spoken of as "influenza." I found that the bronchial and post-nasal sputa were loaded with influenza bacilli; there were associated infections by *micrococcus catarrhalis* and streptococci. Therapeutic immunization was followed by complete cessation of the attacks.

No. 67 was the case of a young man who had been "gassed" in the war. He had ever since suffered from severe bronchitis. I found that his sputum was loaded with influenza bacilli, *streptococcus anginosus* and *micrococcus catarrhalis*. Therapeutic immunization was in this case also completely successful.

No. 3 illustrates the disabling effect of chronic infection. The patient was a naval officer who became depressed and unfit for the performance of his duties. Although there were only very slight catarrhal symptoms, I found that he was suffering from a severe and widespread infection by the bacillus of influenza. Under therapeutic immunization he made a rapid recovery, and was able to resume his duties.

No. 44 was a patient admitted to an asylum on account of acute mania, from several prolonged attacks of which he had previously suffered. I found that his gums were severely infected by the bacillus of influenza, *streptococcus pyogenes* and pseudo-catarrhalis diplococci. He responded almost immediately to therapeutic immunization and made a rapid recovery.

No. 87 was a lady who suffered from severe basal headaches and very occasionally had a discharge of muco-purulent material from the back of the nose. Nasal cultures revealed nothing of importance, but one day the patient was able to supply a fresh specimen of the post-nasal discharge. I found that it was loaded with influenza bacilli. There can be little doubt that the ethmoidal sinuses were involved in this case. Under therapeutic immunization the headaches and intermittent discharge both ceased.

The dose of *bacillus influenzæ* for therapeutic immunization ranges from 0·005 mg. to 0·2 mg. In acute cases, doses of from 0·01 mg. to 0·04 mg. may be given at intervals of twenty-four to forty-eight hours. In cases of chronic infection, treatment should always be begun with a very small dose, because hypersensitiveness is common. Very rarely, cases may be met with in which the heart has been damaged by the bacillary toxin; in these, the effects of minute doses

are often very alarming, and the greatest caution is therefore necessary. The most prominent symptoms of this reaction are severe palpitation and prostration. In ordinary cases tolerance of the toxin is generally rapidly acquired, and, in correspondence with this, there is improvement.

The value of protective inoculation in anticipation of an epidemic has, I believe, been clearly established. Leishman (27), Wynn (29) and others have made observations that seem to prove this fact beyond any doubt. My own experience has satisfied me that the measure is simple, and sufficiently effective to render it of great value. Opinions differ regarding dosage, and also with respect to the other infections against which there should be a simultaneous attempt at immunization. In my opinion (45) it is useless to attempt at the same time to protect against attack by pneumococci and *streptococcus pyogenes*. What it is important to protect against, in addition to the influenza bacillus, is *micrococcus catarrhalis*, which in very many cases prepares the way for the attack by the bacillus. I recommend that a polyvalent emulsion of the following composition should be made up :—

1.cc.= { *Bacillus influenzæ* . . . . 0·2 mg.
Micrococcus catarrhalis . . . 0·2 mg.

At intervals of from two to three days I give successive doses of (1) 0·2 c.c., (2) 0·4 c.c., (3) 0·8 c.c. and (4) 1 c.c. It is generally sufficient to stop at the third dose. The first dose will serve to pick out any case of chronic infection by the severity of the reaction it produces. In such a case, protective inoculation should never be proceeded with. Therapeutic immunization by means of a series of gradually increased doses is alone suitable.

Bacillus pertussis (Bordet-Gengou Bacillus).—On grounds similar to those just adduced with regard to the bacillus of influenza, it has been clearly established that the bacillus of Bordet-Gengou is the cause of the special form of catarrh of the respiratory tract termed "Whooping Cough," which is so common in young children. It is still insufficiently recognized that subacute or chronic infections by the same bacillus occur with some frequency in the adult, causing a persistent catarrh of the respiratory tract characterized

especially by intense irritability of the cough, but rarely accompanied by a whoop. I have observed twelve cases of this kind. They can be distinguished from cases of chronic bronchitis due to other infections only by careful bacteriological examination. The fact is hardly known that acute infection by this bacillus may occur as an epidemic among adults. A clear instance of this occurred in Edinburgh in the spring of 1919. A New Zealand soldier came from his camp in the south of England to get married, prior to returning to his native country. He was suffering from what was regarded as a severe cold with an intensely irritable cough. He made a round of calls with his bride, and afterwards the inmates of nearly every house he visited were attacked by the same type of severe catarrh. In one of these houses, each of three persons in it suffered. I made a bacteriological examination in one of them and found a severe infection by *bacillus pertussis*. There were no influenza bacilli. This case yielded to therapeutic immunization, but only when an associated infection by a pseudo-catarrhalis diplococcus was similarly dealt with. I have no doubt that the infection spread through the city, but as influenza was epidemic at the time, no distinction was made between the two maladies.

The dose of *bacillus pertussis* for therapeutic immunization is about five times larger than that of the bacillus of influenza—that is to say, from 0·02 mg. to 1 mg. In young children, the range is from about 0·01 mg. to 0·4 mg. Details regarding the treatment of whooping cough are given in Chapter XI., section 1.

Protective inoculation against whooping cough is effective and quite practicable. Many observers have now recorded successful results. In view of the high mortality caused by the malady in young children, it is greatly to be deplored that this achievement of science is so rarely applied in practice. The procedure is simple, hardly causes any disturbance and, if generally adopted, would probably save hundreds of lives every year. Three successive doses of 0·02 mg., 0·05 mg., and 0·1 mg. might safely be given to a child between the ages of one and two years. Approximately corresponding doses in millions have been recommended by those who have employed the method extensively.

The Koch-Weeks bacillus is a cause of a form of acute or chronic conjunctivitis. I have had no experience of treatment of cases of the infection, but therapeutic immunization seems to have been applied upon lines similar to those adopted in cases of influenza.

9. Tubercle Bacillus

The results of the extensive observations of many workers seem to warrant the conclusion that pulmonary tuberculosis is in nearly all cases dependent upon infection by the human tubercle bacillus and that a large proportion of glandular, bone and joint tuberculosis is due to infection by the bovine tubercle bacillus. These facts serve as an important guide in the selection of the kind of vaccine, in this instance commonly referred to as *tuberculin*, that we ought to employ.

Much scepticism has prevailed in the past regarding the value of therapeutic immunization in the treatment of tuberculosis. For a long time after its introduction, tuberculin fell into disrepute. In recent years, however, the opinion has certainly been gaining strength that this therapeutic agent can be employed in such a way as to give satisfactory results in most cases. The chief conditions that require to be fulfilled seem to be, that a suitable vaccine be used, that the dose should be one sufficient to produce only a mild reaction, and that the vaccine should be combined with others, either with the object of combating associated infections, or for the purpose of influencing favourably its therapeutic action, as appears to be done by the toxins of *bacillus coli communis*.

Tuberculin is now obtainable in many forms. Probably the most important are the three following :—

1. *Old Tuberculin*, or tuberculin A. (Koch, 1890). It is a boiled and filtered broth culture of the tubercle bacillus and is regarded as an exotoxic tuberculin.

2. *New Tuberculin*, or tuberculin R. (Koch, 1897). This is a solution or emulsion of tubercle bacilli. It is an endotoxic tuberculin.

3. *New Tuberculin, B.E.* (Bacillary Emulsion). This is an emulsion of triturated bacilli.

The first of these is now little used for therapeutic purposes. The choice lies between No. 2 and No. 3, of which various brands are on the market, prepared either from the human or the bovine type of tubercle bacillus. Emulsions of the strength 1 c.c. =0·0001 mg. and 1 c.c. = 0·001 mg. will be found most generally useful, and sufficient to supply the minimum and maximum doses that are likely to be required. My experience with autogenous tubercle bacillus vaccines would seem to show that if these dilutions are made in $\frac{1}{4}\%$ carbolic acid in normal salt solution, the vaccine will remain unimpaired for two years at least. Those who prefer to deal with doses expressed in vulgar fractions will find useful practical advice on the subject in a paper by D'Este Emery (38).

It is a common plan to combine the human and bovine tuberculin in one emulsion for use. In this connection, it is important to remember that the theory is held and strongly supported by evidence (Raw), that human tuberculin has a curative effect on bovine tuberculous infection, and that bovine tuberculin has a curative effect upon the pulmonary form of the disease, which is almost always due to the human type of the tubercle bacillus.

The general principles of dosage already laid down apply equally here. Doses sufficient to produce only mild focal and general reactions must be used. The next dose must be given only after all reactive disturbances have subsided. Focal reactions are manifested by various signs of congestion at the seat of infection, differing with the localization of the disease. In cases of phthisis there is temporary increase of cough and expectoration, and sometimes a little blood in the sputum. Allen recommends the study of the focal reaction by daily auscultation of the chest. A general reaction is indicated by malaise and some rise of temperature. It appears to be possible to regulate the dose in such a way as to produce a distinct focal reaction without any perceptible general reaction, and this nice adjustment of dosage should always be kept in view as an object to be attained. Much valuable advice regarding the regulation of the dose of tuberculin will be found in the writings of Allen (1, 2, 3 and 37), whose extensive experience entitles his practical

recommendations to great respect, and also in a paper by W. H. Wynn (28). Some of the special treatises on tuberculin treatment should also be consulted, such as that of Riviere and Morland (14).

It is probably best to begin with a dose of 0·00001 mg., one-tenth of the initial dose generally recommended. For many weeks very little increase may be tolerated. In favourable cases, a dose of about 0·001 mg. may ultimately be reached. Treatment generally requires to be continued for several months and sometimes for years.

I have treated some cases of tuberculosis with autogenous tubercle bacillus vaccines prepared in the same way as other vaccines, except that about a week is given for sterilization in 1% carbolic acid, instead of one day. That tubercle bacillus vaccines can be satisfactorily prepared in this way has been amply proved by the focal reactions that they have induced and the benefit that has resulted in most of the cases. In one case of phthisis that I treated with a vaccine of this kind, the sputum was tinged with blood always on the third day after the injection, whenever the dose exceeded a certain amount. It was impossible in this case to obtain a record of the temperature, and this slight hæmoptysis served as a correct guide to the dosage, for the patient did well and gained weight, and the bacilli disappeared from the sputum. Only rarely can such a reactive sign be obtained, and its occurrence in this case is cited merely as proof of the possibility of preparing an active autogenous tubercle bacillus vaccine by the ordinary methods of vaccine preparation described in this book. Theoretically, there are great advantages in using an autogenous vaccine, because there are good grounds for believing that there are numerous distinct strains of tubercle bacillus. Some of the failures of tuberculin treatment are probably due to the unsuitability of the tuberculin used for immunization against the patient's strain.

It seems clearly established that the combination of a vaccine prepared from *bacillus coli communis* with tuberculin enhances the therapeutic value of the latter. The action appears to be a pharmacological one, dependent probably from an influence on the vessels. The dose of *bacillus coli*

communis for this purpose ranges between 0·01 mg. and 0·05 mg.

10. Diplococcus Crassus

These Gram-positive diplococci are generally regarded as merely saprophytic bacteria, but there are either several distinctly pathogenic species in the group, or the saprophytic forms are capable occasionally of assuming a pathogenic action. As a saprophyte, it is frequently to be found in the nasal passages, nasopharynx, bronchi and lower genito-urinary tract. The most remarkable case in which I have observed it as a pathogenic agent was one of severe cystitis in an advanced case of tabes dorsalis. The urine, which was alkaline, was loaded with large Gram-positive diplococci. The investigation showed that two distinct types were present, only one of which was capable of splitting up urea, and so of giving a positive reaction in an acid urea test broth.

11. Bacillus Septus

This bacillus appears to be a frequent cause of acute nasal catarrh in some districts. Its occurrence is certainly rare in Edinburgh. On the other hand, nasal catarrh due to infection by a diphtheroid bacillus is common, and I have a strong suspicion that many cases really of this nature have been regarded as being due to *bacillus septus*, an error that might easily have been corrected by testing the bacillus with Neisser's stain.

The therapeutic dose is similar to that of *micrococcus catarrhalis*—namely, from 0·05 mg. to 0·5 mg.

12. Micrococcus Tetragenus

Micrococcus tetragenus and *micrococcus paratetragenus* would seem to occur occasionally as saprophytes, but they are also capable of assuming important pathogenic actions. They are occasionally found along with other generally more important bacteria in the course of the examination of specimens from cases of catarrh of the respiratory tract. Their occurrence is common in phthisical cavities. It is said

that they are also often present in pus from abscesses connected with the teeth. Thornley (*Brit. Med. Journ.*, 21st Oct. 1916) found *micrococcus tetragenus* in the blood in twenty-three out of a hundred cases of "Pyrexia of Indefinite Origin" among the troops in France. The therapeutic dose may be regarded as ranging from 0·05 mg. to 0·5 mg.

13. Bacillus Pyocyaneus

This is one of the important pyogenic bacteria. It may cause abscesses, and is a common infecting agent in wounds, sinuses and ulcers (tubercular, malignant, etc.). It has been found to be a cause of epidemic diarrhœa and wasting in infants. It may be an important infective element in the causation of otitis media. Ernest Hey Grove (*Brit. Med. Journ.*, 15th May 1909) has described a case of septicæmia in which this bacillus occurred in the blood.

The therapeutic dosage is similar to that of the coliform bacillus group, from 0·01 mg. to 0·1 mg.

14. Streptothrices

Streptothrix actinomyces.—Allen (3) has collected the records of fifteen cases of actinomycosis treated with a vaccine, and states that "the results are exceedingly good." The vaccine is difficult to prepare. Wynn (*Brit. Med. Journ.*, 7th March 1908) gives a dose to 0·001 mg.

Anaerobic streptothrices associated with Diabetes Mellitus and Allied Morbid Conditions.—The reader is referred to what has already been stated about these micro-organisms in Chapter VI., section 14. They first came under observation in the course of the study of the bacterial flora in a series of cases of diabetes mellitus, but more recently I have found them in many other cases that were not clinical examples of this disease.

The cases of typical diabetes that have been investigated with regard to the presence of a streptothrix number nineteen, and, in all, a bacterium of this kind was found to be very abundant. I am indebted to Dr Harry Rainy for opportunities of examining eight of these, and to Dr Arthur

Wilson for two cases. In fourteen cases clinically different a similar streptothrix was found. Some of them came under observation before the probable significance of the bacterium was understood, and therefore the urine in these instances was not examined for sugar. In only eight of them was it possible to make a satisfactory examination, and in each instance there was found to be well-marked glycosuria. Every one of these fourteen cases suffered from grave nervous disorders, which included severe neurasthenia, neuritis, paraplegia, insanity with toxic signs (three cases), and dementia præcox (two cases).

I have carried out a number of feeding experiments in rats with this streptothrix, and it was found that they developed glycosuria. Several of them, after living for many months and getting very thin, died after showing symptoms distinctly resembling diabetic coma. In my opinion, the conclusion is justified that, in this streptothrix, we have an important neurotoxic micro-organism of rather common occurrence in the human subject and the cause not only of diabetes mellitus, but also of other grave disorders affecting the nervous system. It causes diabetes, almost certainly, not by its direct action upon starch, but through a selective action upon a particular area of the brain. This action is not always strictly selective and hence the streptothrix becomes a cause, according to the localization of its action, of other organic lesions of the central nervous system. I can recall two cases of acute encephalitis I examined many years ago, both of which showed a micro-organism in the cerebral tissues, morphologically identical with the one under consideration. The pathogenic action of this streptothrix is frequently associated with that of anaerobic diphtheroid bacilli, which may aggravate the neurotoxic condition. I am indebted to Dr Arthur Wilson for opportunities of trying therapeutic immunization against this streptothrix in two advanced cases of diabetes mellitus, but I cannot say that any benefit seemed to be produced. This treatment was also tried in the first case that came under observation, that of a girl of seventeen, and for some weeks there was a reduction in the amount of sugar and improvement in the patient's health ; then there was a relapse and

vaccines were no longer of any avail. The case of a young man treated at the Laboratory gave an encouraging result, for the sugar in the urine diminished in amount and the patient felt so well that he went off to work in the country and was lost sight of. In two other cases in which the treatment was carried out by the practitioner in charge no benefit seemed to result. If diabetes mellitus is dependent upon an organic lesion of the brain, it is easy to understand that therapeutic immunization can be of little avail. Hope lies, I believe, as in pernicious anæmia, in the early detection of the intestinal infection and the suppression of it before it has done irreparable damage.

I recommend an initial dose of about 0·02 mg. Up to 0·4 mg. have been given.

15. Bacillus Lepræ

The use of specific immunization in the treatment of leprosy is considered in Chapter XI., section 7.

16. Bacillus Mallei

The application of therapeutic immunization to the treatment of glanders is dealt with in Chapter XI., section 7.

CHAPTER XI

THE TREATMENT OF INFECTIVE DISEASES BY THERAPEUTIC IMMUNIZATION

In this chapter it is intended to deal with the subject of therapeutic immunization purely from the practical standpoint. As far as possible, all details of the kind that are considered in the preceding sections are excluded. Many infective diseases are omitted for the reason that therapeutic immunization has, at present, no important place in their treatment. The ground that must be traversed is extensive, and, with very occasional exceptions, no attempt is made to cover that of symptomatology. The arrangement of subjects is merely one of convenience, and some are admittedly misplaced, if their position is to be judged strictly by the localization of the infection. For example, the various forms of rheumatism are dealt with under diseases of the bones and joints, but they are really due to bacterial infections of certain mucous membranes; similarly, many forms of insanity are dependent upon the action of bacterial toxins that have their source in distant non-nervous tissues.

1. Diseases of the Respiratory Tract

These are considered in the order indicated in the following classification, which is simply one of convenience:—

(a) *Acute Infections of the Upper Respiratory Tract and of the Whole Tract*

1. Common Colds.
2. Acute Influenza.
3. Whooping Cough.
4. Acute Infections of the Eustachian Tubes. Acute Otitis Media.

(b) *Chronic Infections of the Upper Respiratory Tract*

5. Recurrent and Chronic Nasal Catarrhs. Ozæna.
6. Infections of the Accessory Sinuses of the Nose.
7. Chronic Post-Nasal Catarrh.
8. Chronic Infections of the Eustachian Tubes. Otitis Media. Progressive Deafness.
9. Hay Asthma.

(c) *Acute Infections of the Lower Respiratory Tract*

10. Acute Bronchitis and Laryngitis.
11. Acute Lobar Pneumonia.
12. Acute Catarrhal Pneumonia.

(d) *Chronic Infections of the Lower Respiratory Tract*

13. Chronic Bronchitis.
14. Asthma.
15. Phthisis.

Absolute distinctions between diseases of the upper respiratory tract and those of the lower cannot always be made. For example, in a common cold the infecting bacteria may attack only the upper respiratory tract, only the lower portion, or the whole tract.

Much important information, and valuable advice founded on very extensive experience, are given in Dr Allen's *Bacterial Diseases of Respiration* (2), and in his series of articles on the same subject in the *Journal of Vaccine Therapy*, 1912.

A word requires to be said about the bacterial flora of the respiratory tract in health. The nasal mucus, representing the anterior nasal passages and sinuses, may be sterile, or it may yield a few colonies of a mannite non-fermenting staphylococcus, of non-pathogenic diphtheroid bacilli, or of pseudo-catarrhalis diplococci. The nasopharynx, on the other hand, always contains abundant bacteria, for the most part saprophytes. In addition to those just mentioned as commonly occurring in the anterior nasal passages, streptococcus mucosus and streptococcus salivarius are the most common. A few colonies of pathogenic bacteria such as *streptococcus*

pyogenes, *streptococcus anginosus* and *staphylococcus pyogenes* may occasionally be found. We judge of the occurrence of a local bacterial attack from the presence of enormous numbers of pathogenic bacteria, and the evidence of local inflammatory action. The testimony of focal reaction and successful therapeutic immunization have been added in a very large number of cases to confirm the accuracy of this inference.

1. **Common Colds.**—That epidemic catarrhs of the respiratory tract, or common colds, are of bacterial origin is now established beyond serious question. The part played by chill, by no means essential, is merely that of lowering resistance to attack, and permitting a latent infection to become an active one. The pathogenic species that may attack are numerous, and most of them have already been recognized and described by various workers. One of the most important early papers was that of Benham (20). The bacterium that is responsible for the majority of acute infective catarrhs of this kind is *micrococcus catarrhalis*, of which there are several independent epidemic strains. Other frequent causes are *bacillus influenzæ*, a catarrhal diphtheroid bacillus, pneumococci and *bacillus septus*. Some of the bacteria that more rarely occur as causes of infective catarrh of this kind are the bacillus of Friedländer, *staphylococcus pyogenes*, *bacillus proteus*, *streptococcus equinus*, *streptococcus anginosus* and *streptococcus pyogenes*. There is also to be mentioned here, as an infection of the adult, *bacillus pertussis*.

Whilst there is nearly always one leading and essential infective agent in these cases, combined attacks are common, such as, by *micrococcus catarrhalis* and the bacillus of influenza, and by *micrococcus catarrhalis* and pneumococci. The acute attack by the new infective agent generally stimulates chronic or latent infections of other kinds that may happen to be present. Thus, whilst the long-chained streptococci can only rarely be regarded as causes of infective colds, they commonly take part in an acute attack if they already occur as chronic or latent infective agents.

I have made a bacteriological investigation in 50 cases of common cold. From these I have excluded all cases of typical acute influenza, and of whooping cough in children. A large proportion of them occurred in patients undergoing

therapeutic immunization for chronic infections of some part of the respiratory tract. In these cases, the previous flora of the tract was known and with the attack of acute catarrh, a new infective element was always found to have been introduced. The casual relationship of the infection to the acute illness was in many instances established by the occurrence of focal reactions to an autogenous vaccine and rapid recovery under immunization. The infective causes of the acute catarrh in these 50 cases appeared to be *micrococcus catarrhalis* in 23, a catarrhal diphtheroid bacillus in 9, bacillus of influenza in 8, a pneumococcus in 5 (in two of these *micrococcus catarrhalis* was equally prominent), *bacillus septus* in 2, and *bacillus catarrhalis, bacillus pertussis* (in an adult), *streptococcus equinus, bacillus proteus,* bacillus of Friedländer, *streptococcus anginosus* and *staphylococcus pyogenes aureus* in 1 each. It is of considerable importance to understand that *micrococcus catarrhalis* does not represent one species but includes several, and that immunization against one of them does not necessarily protect against the others. *Bacillus catarrhalis* must be included as one of the possible causes of acute coryza, but the anaerobic forms of *micrococcus catarrhalis* do not appear to cause epidemic catarrhs. The occurrence of a form of infective cold, due to the pathogenic action of the catarrhal diphtheroid bacillus is hardly recognized. There are probably several independent strains. A bacillus of this kind was a common cause of a subacute type of coryza in Edinburgh in the winter of 1919. The infection produces a general aching all over the body, not unlike that of influenza ; there is commonly congestion and swelling of the nasal passages, nasopharynx and palate, accompanied by a hot, dry feeling and dull pain in this region. This pain may extend in the form of headache. In the early stages, there is only slight increase of the mucous secretion, and coughing and sneezing are not very prominent symptoms. There may be no rise of temperature. There is little tendency of the infection to spread to the lower respiratory tract.

The bacillus of influenza may induce a catarrh of the respiratory tract that is clinically indistinguishable from a

common cold caused by other bacteria, and it must therefore be included in the causes of this malady. The importance of pneumococcus infections as a cause of common colds is not sufficiently understood. An infection of this kind is probably the initial stage of many cases of pneumonia. *Bacillus septus* colds, whilst apparently common in and around London, are certainly comparatively rare in the north. There is strong reason to believe, however, that a catarrhal diphtheroid bacillus has often been mistaken for *bacillus septus*. I have observed cases that have clearly established that infections by *bacillus proteus*, *staphylococcus pyogenes aureus* and *streptococcus equinus* may cause attacks of acute coryza. *Streptococcus anginosus* and *streptococcus pyogenes* are not to be regarded as ordinary causes of epidemic colds. I have, however, seen one case in which infection by the two together was apparently conveyed from another person. The bronchial flora of this patient had previously been examined and neither of these two species was present, prior to the acute attack which was clearly due to them.

A common cold can generally be cut short by appropriate therapeutic immunization, but the uncertainty of the exact bacterial cause in some cases creates serious practical difficulties. In severe epidemics due to a particular strain of *micrococcus catarrhalis*, it is, however, possible to prepare a vaccine that is suitable for nearly all cases. In other circumstances, a polyvalent vaccine often succeeds very well. An emulsion of the strength 1 c.c. equals 0·2 mg. is convenient. The initial dose should be from 0·02 mg. to 0·04 mg. In the acute stage, doses may be given at intervals of from twenty-four to forty-eight hours, the amount being increased each time. It will rarely be necessary to go above 0·2 mg. unless it is desired, after the infection has been suppressed, to carry out protective inoculation, in which case a tolerance of at least 0·5 mg. should be attained.

The therapeutic dose of the catarrhal diphtheroid bacillus ranges from 0·02 mg. to 0·5 mg.

In view of the impossibility of having a bacteriological investigation made in every case of common cold, it has become customary to use compound vaccines containing the principal causal bacteria in suitable amounts. A polyvalent

vaccine of this kind that I have frequently supplied has the following composition :—

1 c.c. = { *Bacillus influenzæ* . . . 0·1 mg.; *Micrococcus catarrhalis* . . 0·2 mg.; *Pneumococcus* . . . . 0·05 mg.; *Streptococcus pyogenes* . . 0·05 mg. }

The initial dose is from 0·1 c.c. to 0·2 c.c. *Streptococcus pyogenes* is included because of the frequency with which this important pathogenic bacterium, occurring as a latent infection, invades the mucous membranes especially of the nasopharynx, fauces and lower respiratory tract when these regions are the seat of acute catarrh induced by other infections. Catarrhal diphtheroid bacillus, 0·2 mg., and *streptococcus anginosus*, 0·05 mg., might suitably be added to the above vaccine, but I have given the composition that I find is commonly desired by practitioners.

There is a considerable amount of evidence that much can be done to prevent the occurrence of common colds by protective inoculation against *micrococcus catarrhalis*. The real difficulty is to protect against all the strains to which the person may be exposed. Many patients who have had protective inoculation against influenza with a vaccine that included *micrococcus catarrhalis* have reported to me that they remained free from attacks of common cold for several months, whereas ordinarily they would have suffered from one or more colds. A compound vaccine of the above composition may be used for this purpose.

Three or four injections should be given at intervals of about four days beginning with 0·2 c.c. and rising to 2 c.c. The only limit to the dose ultimately reached is the tolerance shown by the patient, and closely in proportion to his tolerance of the bacterial toxins will be his power to resist attack by the corresponding bacteria.

2. **Acute Influenza.**—In Chapter X., section 8, reasons have already been given for believing that *bacillus influenzæ* is the cause of epidemic influenza, notwithstanding the numerous attempts that have recently been made to discredit its relationship to the disease.

In the experience of many doctors, therapeutic immuniza-

tion is of great service in the treatment of acute influenza. My own observations, though not very numerous, bear out this conclusion. In several cases, in which the presence of the bacillus was ascertained by bacteriological investigation, the administration of small doses of polyvalent vaccine was quickly followed by fall of temperature and rapid recovery. The question arises whether we should combine the influenza bacillus vaccine with other vaccines. I think the facts warrant only an affirmative answer. Along with the attack by the bacillus of influenza, other pathogenic bacteria, already present at the surface, commonly invade the tissues and aggravate the illness ; therefore it is important to endeavour to stimulate the defences against the latter also. The most important secondary infections of this kind are those by *micrococcus catarrhalis, streptococcus pyogenes* and pneumococci. A suitable combination is therefore the compound catarrhal vaccine, the formula of which has been given in the preceding subsection. Whether we give a polyvalent bacillus of influenza vaccine, or a compound one, the dose should always be small. I think it is generally advisable to repeat the dose every twenty-four hours, though some prefer to make the interval not shorter than forty-eight hours. A first injection of *bacillus influenzæ* 0·02 mg., *micrococcus catarrhalis* 0·04 mg., *pneumococcus* 0·01 mg. and *streptococcus pyogenes* 0·01 mg. may be given. The next dose should be the same, or of double the amount of each element. It will not generally be found necessary to increase the dose much beyond this.

Protective inoculation against influenza has already been dealt with in the preceding chapter.

3. **Whooping Cough.**—Although whooping cough is commonly regarded as only a disease of children, it is of considerable practical importance to bear in mind that acute and chronic infections by *bacillus pertussis* occur with some frequency in adults, in whom the real nature of the disease is apt to pass unrecognized, because the most distinctive symptom is not whooping, but an extremely irritable cough. In all cases in the adult with this symptom, suspect either a pneumococcus infection, or one by the bacillus of whooping cough.

There are some cases of whooping cough in children in which the most careful search fails to reveal the presence of *bacillus pertussis*. Only diplococci of the catarrhalis group, or these and pneumococci, or *streptococcus pyogenes*, can be found. Two explanations are possible: First, that, in some children, the irritation caused by the toxin of these bacteria is capable of producing a spasmodic cough, and second, that there was an infection by *bacillus pertussis* at an early stage of the malady, but it became suppressed, and infections by other catarrhal bacteria took its place, whilst the characteristic hypersensitiveness remained and the cough therefore retained its special feature.

It has been established that whooping cough can be treated successfully, in most instances, by therapeutic immunization. It is, however, even more important than in influenza to take into account the secondary infections to which the high mortality of the disease is due. These secondary infections vary from case to case and hence it is impossible, without a bacteriological investigation, to know exactly what bacterial enemies we have to fight. Nevertheless, experience has shown that it is of advantage to combine the specific vaccine with doses of *micrococcus catarrhalis*, *pneumococcus*, *streptococcus pyogenes* and the bacillus of influenza. It is of the utmost importance to understand that the dose of *bacillus pertussis* is about five times that of *bacillus influenzæ*. Success in treatment, in adults at least, is dependent upon pushing the dose to a proper amount, which will not be reached if it is supposed that it is the same as that of Pfeiffer's bacillus.

The following is the formula of a vaccine that I am accustomed to supply for use in young children :—

1 c.c. =

Bacillus influenzæ . . .	0·02 mg.
Bacillus pertussis . . .	0·1 mg.
Micrococcus catarrhalis . .	0·1 mg.
Pneumococcus	0·02 mg.
Streptococcus pyogenes . .	0·02 mg.

The initial dose of this vaccine is 0·1 c.c. The maximum, to be reached only after five or six gradually increased doses have been given, is about 1 c.c. The intervals between the doses should be from three to four days.

In the treatment of the disease in adults, a complete bacteriological examination should always be made, and a compound vaccine prepared in accordance with the results. For patients of this kind, the dose ranges from 0·05 mg. to 0·5 mg.

Evidence that has now been brought forward by many observers shows that protective inoculation is easily carried out and effective. In view of the great frequency with which the complications of whooping cough prove fatal, it is greatly to be deplored that protective inoculation is not more largely used. Three or four graduated doses ranging from 0·01 mg. to 0·1 mg., or more, according to the age of the child, should be given at intervals of three or four days.

4. **Acute Infections of the Eustachian Tubes. Acute Otitis Media.**—These very common maladies are generally dependent upon an extension of bacterial infection from the nasopharynx, and they may therefore conveniently be considered here. The bacteria concerned are of numerous species, and mixed infections are frequent. *Streptococcus pyogenes*, *staphylococcus pyogenes* and *pneumococci* seem to be the most common invaders; others, found less frequently, are *micrococcus catarrhalis*, *bacillus influenzæ*, *bacillus coli communis*, *bacillus proteus*, *bacillus typhosus*, bacillus of Friedländer, *bacillus pyocyaneus*, *bacillus diphtheriæ* and meningococcus. Although several observers have recorded good results from the use of vaccines, the methods of treatment employed seem rarely to include this measure. This fact is greatly to be deplored, as much good can be done and grave complications may be avoided by prompt and accurate immunization. It is essential to ascertain the flora of the discharge and to prepare autogenous vaccines. Two of the following cases illustrate the successful application of therapeutic immunization in this disease.

A student, after suffering for some days from a common cold, developed all the symptoms of acute catarrh of the Eustachian tube and tympanum on one side. Paracentesis became necessary, and a specimen of the pus was sent to me for examination. It yielded profuse growths of *streptococcus pyogenes* and of *staphylococcus pyogenes albus*. A swab taken from the entrance to the Eustachian tube yielded the same flora. The patient was treated with autogenous

vaccines, frequently administered in small doses, and made a rapid and complete recovery.

In another, but more advanced case, occurring in a boy, pus discharged through the perforated tympanic membrane gave colonies of *streptococcus pyogenes* and *staphylococcus pyogenes aureus*, but pus taken from the mastoid cells at operation on the following day yielded only the former. In another case in a young girl, pus from the middle ear yielded colonies of *staphylococcus pyogenes albus* and *streptococcus pyogenes* in proportion of 4 to 1. This case was treated by immunization and made a rapid recovery.

The clear indication in such cases is to carry out as quickly as possible a bacteriological investigation, to prepare autogenous vaccines, and to treat by small doses, repeated at first every day, and, later, every second day.

5. **Recurrent and Chronic Nasal Catarrhs. Ozæna.**—Chronic nasal catarrh, aggravated from time to time by acute exacerbations, is an extremely common malady. Much less common, but still frequently to be met with, is recurrent nasal catarrh marked by repeated attacks, but with freedom from all symptoms between these attacks. Both types are chiefly dependent upon chronic bacterial infection of the nasal mucosa.

Attempts are frequently made to belittle the importance of bacteria as causes of chronic nasal catarrh. It is alleged that the bacteria demonstrated by the bacteriologist are merely those normally present that have multiplied in consequence of perversion of the mucous secretion. Such a contention is contrary to the facts established by very extensive observation and experience. *Micrococcus catarrhalis, streptococcus pyogenes, pneumococci, staphylococcus pyogenes* and the bacillus of Friedländer, which are among the bacteria most commonly found in cases of this kind, are not normal inhabitants of the nasal passages. Systematic bacteriological investigation of cases in health as well as in morbid states, the observations of focal reactions, and the successful results of therapeutic immunization directed in accordance with the features of the pathogenic flora of the case, prove that bacterial infection is the chief cause of nasal catarrh and that it is operative even in most cases in which a mechanical or chemical irritant (for example, the pollen of grasses) is an obvious factor.

The bacteria that may cause recurrent and chronic nasal catarrh are numerous. Any one of those associated with acute epidemic coryza may become established as the agent of a chronic infection, and there are others to be added to the list, such as mannite non-fermenting staphylococci, numerous types of diphtheroid bacillus, *bacillus fæcalis alcaligenes*, *bacillus coli communis*, *streptococcus fæcalis hæmolyticus*, *diplococcus crassus* and *micrococcus paratetragenus*. Mixed infections are the rule, and the symptoms vary from case to case in accordance with differences in the flora and the localities specially involved. It is never safe to conclude what the infective causes are in any case without the evidence of a bacteriological examination. The most common infecting bacteria are *micrococcus catarrhalis*, *staphylococcus pyogenes*, mannite non-fermenting staphylococci, diphtheroid bacilli, *bacillus influenzæ*, *streptococcus pyogenes* and *pneumococci*. Involvement of the accessory sinuses is frequent and often a very troublesome complication. One of the consequences of chronic bacterial infection of the nasal mucosa in some persons is the development of polypi.

Treatment by therapeutic immunization should never be undertaken without a previous thorough bacteriological investigation. Every pathogenic species found should be included in the vaccines. When treatment has continued for five or six weeks, a supplementary examination should generally be made. Bacteria represented only by delicate colonies that were obscured by larger colonies at the first examination and so escaped observation may then often be found. The presence of polypi is generally attended by hypersensitiveness to autogenous vaccines. In some cases this cause may be overcome by repetition of minute doses, but in most the hypersensitiveness persists. I have observed its immediate disappearance after the polypi have been removed by a surgeon. As a rule therapeutic immunization in cases of chronic infection of the nasal passages cannot be successful unless existing polypi are removed.

Chronic nasal catarrh associated with syphilis, tuberculosis, lupus and glanders hardly call for consideration in this section. Ozæna is generally dependent upon chronic infection

by an aberrant type of the bacillus of Friedländer. This fact has been clearly brought out by Abel, who studied a hundred cases (see Allen (1), p. 341). Foul-smelling nasal discharge may, however, be produced by the action of other bacteria, especially *bacillus proteus*, *bacillus coli communis*, *bacillus pyocyaneus* and *staphylococci*.

I have investigated and treated several cases of the Friedländer type. The following less common type may be of interest :—

A nurse had suffered for many years from ozæna. Bacteriological examination of the nasal discharge revealed a severe infection by *bacillus proteus*. *Streptococcus pyogenes* and a mannite non-fermenting staphylococcus were also present. The patient made an almost complete recovery under therapeutic immunization. Slight chronic catarrh remained, but the fœtor had gone. A second examination, made nine months after the first, showed that all of the bacteria against which immunization was directed had disappeared; the cultures yielded a few colonies of *staphylococcus pyogenes aureus*, a minute diphtheroid bacillus and a pseudo-catarrhalis diplococcus.

6. **Infections of the Accessory Sinuses of the Nose.**—The maxillary, frontal, ethmoidal and sphenoidal sinuses may be attacked by bacteria in the course of acute or chronic infection of the nasal passages. *Bacillus influenzæ*, *micrococcus catarrhalis*, *staphylococcus pyogenes*, *streptococcus pyogenes*, *pneumococci* and diphtheroid bacilli are probably the most common invaders. It is important to remember that such infections may sometimes lead to optic neuritis. Numerous cases in which this occurred have been described by Henry Manning Fish (*Brit. Med. Journ.*, 2nd Nov. 1907).

The Maxillary Sinus (*Antrum of Highmore*) is perhaps the one most subject to chronic bacterial attack. The infections are usually mixed and they vary in character from case to case. Thus in four cases that I have investigated, the pathogenic bacteria found were (1) *streptococcus pyogenes* and *bacillus influenzæ*, (2) *streptococcus anginosus* and *pneumococcus*, (3) *streptococcus pyogenes*, mannite non-fermenting staphylococci and a streptothrix, and (4) *staphylococcus pyogenes albus*, *micrococcus catarrhalis* and an anaerobic diphtheroid bacillus. The patients are generally benefited by accurate therapeutic immunization, but on

account, probably, of thickening of the mucosa and defective drainage of the cavity, it is often impossible to obtain a satisfactory result by this measure alone.

The Frontal Sinuses are often involved in acute nasal catarrhs. Cases of chronic infection would appear not to be very common.

Ethmoidal and Sphenoidal Sinuses.—I have investigated three cases in which involvement of the ethmoidal sinuses was diagnosed by a nasal specialist. In the first, cultures from the discharge yielded abundant growths of *bacillus influenzæ, pneumococci, micrococcus catarrhalis* and *staphylococcus pyogenes albus.* In the second, the infecting bacteria were *bacillus influenzæ, diplococcus crassus* and *streptococcus pyogenes.* In the third, *staphylococcus pyogenes aureus* and *micrococcus catarrhalis* were found.

7 **Chronic Post-nasal Catarrh.**—For reasons that do not fully appear, the post-nasal space is more prone probably than any other region of the body to break down in its defences against attack by common pathogenic bacteria. I have investigated 153 cases. Infections of this region may give rise to rheumatism, and to rheumatoid arthritis; they may extend to the bronchi, producing acute or chronic bronchitis and pneumonia, or to the alimentary tract, giving rise to gastric catarrh, gastric or duodenal ulcer and various chronic infective diseases of the intestine, such as chronic pneumococcal enteritis.

A very large variety of bacteria may attack the nasopharynx. The infections are always mixed. *Streptococcus pyogenes, streptococcus anginosus, streptococcus fæcalis hæmolyticus, micrococcus catarrhalis, pneumococci, staphylococcus pyogenes* and the bacillus of influenza are the pathogenic bacteria most commonly to be found. Among others that may occasionally be present are the bacillus of Friedländer, *bacillus fæcalis alcaligenes, bacillus proteus* and diphtheroid bacilli.

As a preliminary to therapeutic immunization, it is essential that there should be made a careful analysis of the flora of a post-nasal swab, or of sputum derived from the nasopharynx. It is well to examine both. All pathogenic bacteria found to be present should be included in the vaccine. A supple-

mentary examination should be made after some weeks, and, if any pathogenic species remain, fresh vaccines should be prepared from them and immunization continued. In view of the havoc wrought in the health of children, as well as of adults, by chronic post-nasal infections, it is greatly to be deplored that they are so rarely investigated bacteriologically and treated by immunization.

8. **Chronic Infections of the Eustachian Tubes. Chronic Otitis Media.**—The extension of chronic infections of the nasopharynx to the Eustachian canal and tympanum is unfortunately very common. Acute infections have already been considered. Chronic infections are much more frequent, and they generally entail progressive deafness. In view of the inadequacy, in very many cases, of orthodox treatment as a means of arresting the natural course of the malady, it is important that the claims of therapeutic immunization, based on accurate bacteriological investigation, should be fully considered.

About 10% of the 153 cases of chronic post-nasal catarrh that I have investigated suffered from deafness. It is generally impossible to establish by direct bacteriological methods that there is an invasion of the Eustachian tube by bacteria. There are, however, indirect methods of obtaining information. If the Eustachian tube is invaded by bacteria, the same bacteria are with certainty contained in the mucous membrane of the nasopharynx. We can take swabs from the region of the orifice of a Eustachian tube and analyse the flora and prepare vaccines. If, in response to injection of suitable doses of one or other of these vaccines, the patient experiences temporary increase of deafness, local discomfort and pain, and noises in the ears, we have evidence of a focal reaction having occurred in the canal, and proof that the corresponding bacterium is attacking its walls. This method of ascertaining the infective causes of chronic middle ear disease appears to be unknown to the aural surgeon, one of whom I recently heard deplore before an important medical congress our entire lack of knowledge of the essential causes of chronic middle ear disease and progressive deafness. I have observed in very many cases this phenomenon of focal reaction in catarrh of the Eustachian tube and have confirmed

the conclusion based upon this reaction by successful therapeutic immunization.

The discharge from cases in which there is a perforated tympanic membrane always yields profuse growths of pathogenic bacteria. In such cases it is open to the unbeliever in therapeutic immunization to maintain that the bacteria are accidental invaders from outside. This may occasionally be true, but it often accords ill with the nature of the flora, and it has in many cases been sufficiently refuted by the observation that the flora of a swab taken from the nasopharynx and that of pus discharged from the ear were identical. Moreover, in such cases, therapeutic immunization based upon the flora of the discharge has generally proved successful.

The cases that I have investigated have appeared to show that the most important infections associated with chronic non-suppurative forms of middle ear disease are by *streptococcus pyogenes*, *bacillus influenzæ*, *micrococcus catarrhalis* and various types of diphtheroid bacillus. In chronic suppurative cases with discharge from the ear, *staphylococcus pyogenes*, *streptococcus pyogenes* and *bacillus proteus* have been the chief infective agents.

It may be laid down as a rule that persons suffering from progressive deafness have chronic post-nasal catarrh extending into the Eustachian tubes and that they benefit from therapeutic immunization accurately directed against the bacterial causes of this catarrh. There is a residuum of cases in which the sclerotic changes in the middle ear are dependent, not upon invasion of the Eustachian tube, but upon a bacterial toxic action very similar to that which causes rheumatic changes in joints. In this instance, the infective focus is in the nasopharynx, anterior nasal passages, fauces, gums or elsewhere, and treatment should include suppression of any such infective foci by therapeutic immunization. The first indications of progressive deafness should be the signal for the making of a thorough bacteriological investigation of all possible seats of infection, including the orifices of the Eustachian tubes. If any bacteria are found to be present in large numbers, autogenous vaccines should be prepared and the patient given a course of therapeutic

immunization. A supplementary bacteriological investigation should always be made towards the end of the course.

9. **Hay Asthma.**—This very painful malady, which recurs in some people with unfailing regularity every summer, is generally regarded as sufficiently explained by hypersensitiveness of the nasal mucosa to the pollen especially of certain grasses. The fact that immunization against the pollen of *Phleum pretense* has been found to protect many cases from attack seems on first view to confirm this conclusion. Other victims of hay asthma have, however, been similarly cured by suppression of chronic bacterial infection of the nasal passages. The truth seems to be that the special hypersensitiveness to pollen is largely dependent upon an abnormal state of the nasal mucosa caused by the action of bacterial toxins in persons in whom no doubt there is a certain special inherent predisposition to such hypersensitiveness, because this certainly does not occur in more than a very small proportion of those who suffer from chronic nasal infections. Some influence must be allowed, however, to the kind of chronic infection that is present. The fact remains that experience has shown that, if existing nasal infections are carefully investigated and eradicated by immunization, the patient passes through the period when the air is laden with pollen dust without suffering from hay fever. The inference from this is that the proper preventive treatment of hay asthma consists in the suppression of chronic nasal infections. If we depend alone upon creating an artificial tolerance of the toxin contained in the pollen, the nasal infections remain and the patient is still subject to the many inconveniences and dangers that these very commonly entail.

10. **Acute Bronchitis and Laryngitis.**—The infective causes of these maladies are simply those of acute catarrhs of the upper respiratory tract, but with a relatively much greater incidence of invasion by the long-chained streptococci and pneumococci. These and *bacillus influenzæ, bacillus pertussis, micrococcus catarrhalis, streptococcus fæcalis hæmolyticus, staphylococcus pyogenes* and the bacillus of Friedländer are the pathogenic bacteria most commonly found in the sputum.

Therapeutic immunization should be applied in accord-

ance with the principles already indicated for acute infections. Pending completion of a bacteriological investigation and the preparation of autogenous vaccines, a stock vaccine of the composition of that recommended for common colds is suitable.

11. **Acute Lobar Pneumonia.**—As the causal agent of acute lobar pneumonia chief importance must now be attached to *pneumococcus*, Type I. Types II. and III. seem to be less frequent causes of the disease. It is to be borne in mind, however, that there are other bacterial causes of acute pneumonia, among which there are especially the bacillus of influenza, *streptococcus pyogenes*, and the bacillus of Friedländer.

Until recently, therapeutic immunization has not been thought of any value as a means of treatment. Many observers have now, however, obtained results that have seemed to them to justify the conclusion that early vaccine treatment is attended with a considerable measure of success. The nature of my official work has precluded me from opportunities of obtaining any personal experience of the treatment of cases of this kind by vaccines, and I have therefore to rely entirely upon the recorded experiences of others. In accordance with the principles that it has already been maintained ought to guide us in the application of therapeutic immunization to acute infective diseases, lobar pneumonia should be one of the maladies amenable to this form of treatment. Accurate work is essential. There must be a correct bacteriological diagnosis, and an autogenous vaccine should, as soon as possible, be substituted for the stock one with which we are generally obliged to begin.

Allen (1) deals fully with the subject. He directs that cultures be made immediately from the sputum with the object of preparing an autogenous vaccine and that treatment in cases of pneumococcus infection should be begun immediately with a dose of 25,000,000 of a polyvalent stock vaccine. If there is no "response" (that is, if there is no improvement in the patient's condition) after thirty-six to forty-eight hours, this dose is to be repeated, the autogenous vaccine being used if it is ready. If there is still no response after thirty-six to forty-eight hours, double the dose is to be

given. If there is response, "defer re-inoculation for three days." He gives a résumé of the results obtained by other workers.

M. H. Gordon, Butler Harris (33), Wynn (30) and Henry A. Craig (25) have also recorded favourable results with pneumococcus vaccines.

Protective inoculation against acute pneumococcus infection has been successfully used by Wright and Lister in South Africa, by workers in the Rockefeller Institute (34), Cecil and Austin (36), Allen (3) and others. As Allen very rightly remarks, very serious consequences may result from giving large doses for this purpose to persons who are already suffering from chronic pneumococcus infections. The only way of guarding against this danger is to make the initial dose a very small one Nevertheless, it has been recommended (34) that an initial dose of 1,000,000,000 of each strain (Types I., II. and III.) should be given, a second of 1,000,000,000 or 2,000,000,000 after seven days, and a third of 2,000,000,000 after a similar interval.

12. **Acute Catarrhal Pneumonia.**—The infective causes of catarrhal pneumonia are identical with those of acute bronchitis, of which it is an extension, and the indications for therapeutic immunization may be regarded as also the same.

13. **Chronic Bronchitis.**—Any of the pathogenic bacteria already mentioned as causes of acute or chronic infection of the upper respiratory tract may take part in an infection of the bronchi, to which chronic bronchitis is mainly due. The action of chemical irritants is chiefly that of damaging the mucosa so that it becomes more easily attacked by bacteria. The Great War has left hundreds of cases of chronic bronchitis that began through the patient being gassed. I have investigated the sputum in many cases of this kind and have always found that there was a profuse pathogenic flora. Every case treated by therapeutic immunization has made a satisfactory recovery. The obliviousness of army medical officers and pension officials to the possibility of curing these cases by this means is much to be regretted, for it entails to hundreds much suffering that might be relieved.

The chief bacterial causes of chronic bronchitis are *strepto-*

coccus pyogenes, streptococcus anginosus, micrococcus catarrhalis (aerobic and anaerobic), *bacillus influenzæ, bacillus pertussis, staphylococcus pyogenes, streptococcus fæcalis hæmolyticus*, the bacillus of Friedländer, and various diphtheroid bacilli. As a preliminary to treatment by therapeutic immunization, a very careful bacteriological analysis of the sputum is necessary. Immunization should be carried out against every pathogenic bacterium found. Separate vaccines should be prepared in suitable dilutions and the dose of each regulated in accordance with the principles already laid down. It is important to give doses that produce only mild focal reaction. If hypersensitiveness is revealed, the dose should immediately be reduced to about one-tenth. Allen rightly insists upon the importance of treating radically any co-existing oral or throat sepsis.

The use of stock vaccines in cases of chronic bronchitis cannot be regarded as satisfactory, because the flora differs greatly from case to case, and it is impossible to be sure of its exact nature without a bacteriological investigation. As a rule, the use of stock vaccines in this malady will lead to disappointment.

A medical student who had served as an officer in the army during the war, and had been severely gassed, had ever since suffered from chronic bronchitis with occasional exacerbations. Cultures made from the sputum yielded profuse growths of *streptococcus pyogenes, micrococcus catarrhalis* and diphtheroid bacilli. The bacillus of influenza was also present. Under therapeutic immunization against these infecting bacteria, he made a complete recovery.

Another medical student with a similar history was found to have severe bronchial infections by pneumococci and *streptococcus pyogenes* and a post-nasal infection by *micrococcus catarrhalis*. A course of therapeutic immunization with autogenous vaccines resulted in cessation of all bronchitic trouble.

A young soldier had suffered for many years from chronic bronchitis. He had to be sent home from service in France, and was given light duties in Edinburgh. An officer to whom he had been appointed to act as batman asked me to undertake his case. No doctor in the army under whose care the patient had come had ever suggested a bacteriological investigation and treatment by therapeutic immunization. I found that he had very severe bronchial infections by pneumococci, *streptococcus fæcalis hæmolyticus* and *micrococcus catarrhalis*. He responded splendidly to treatment with autogenous vaccines and made a complete recovery.

14. **Asthma.**—Bronchial asthma is almost always associated with chronic infection of the lower respiratory tract. The pathogenic flora does not differ essentially from that of chronic bronchitis; asthma is an individual reaction. In some cases, the infective foci are chiefly in the upper respiratory tract, and in these mechanical obstruction from polypi often aggravates the trouble. All attempts to attribute asthma specially to any particular bacterial species (such as *streptococcus pyogenes*) break down before the evidence of a long series of cases investigated bacteriologically. Bacterial infection and mechanical obstruction are not the only essential pathogenic factors; the patient is a person with a special nervous predisposition to asthmatic reaction. Errors of diet and indigestion may aggravate asthma, and various odours and even strong mental impressions may pull the trigger and determine an asthmatic explosion.

Most cases do well under therapeutic immunization, if the bacteriological investigation is complete, and all pathogenic species are included in the autogenous vaccines. An attack of asthma is generally one of the manifestations of focal reaction when a full dose is given. Many cases give trouble on account of hypersensitiveness and consequent violent focal reactions that may last for several days. The proper way to deal with such cases is to reduce the dose to one-tenth of the ordinary initial one. When this is done, and the vaccine repeated every three or four days, the patient generally improves at once.

I have no experience of peptone immunization in the treatment of asthma, but I would protest against reliance being placed upon this measure alone. Its advocates ignore the fact of chronic infection of the respiratory tract. If the peptone immunization relieves the asthma, as it has been alleged to do in some cases, the chronic infections remain. Nothing has been done to suppress them. If the chronic infections of the respiratory tract are cured, the patient will get rid at once of his chronic bronchitis, or rhinitis, and of his asthma also.

A boy of fourteen had suffered from chronic bronchitis and repeated attacks of asthma for seven years. He was under-developed and his education was being seriously retarded owing to his inability to

attend school with any regularity. I found that he had severe post-nasal and bronchial infections by *streptococcus pyogenes*, *staphylococcus pyogenes aureus* and *micrococcus catarrhalis*. Under therapeutic immunization his symptoms gradually disappeared, and he remained well for a time, but relapsed after about a year. Another investigation revealed evidence of infection of the respiratory tract by pneumococci, *streptococcus pyogenes*, *streptococcus anginosus*, *streptococcus fæcalis hæmolyticus*, and *micrococcus catarrhalis*. He responded better than on the first occasion to immunization, and made a complete recovery. From about the time of the completion of the first course of immunization, he began to grow rapidly, and in the course of two years had developed into a sturdy youth. He was able to attend school regularly and soon recovered the ground he had lost.

A gentleman past middle age had suffered for many years from frequently recurring attacks of bronchial asthma. Bacteriological examination of his sputum showed evidence of bronchial infection by pneumococci, *streptococcus pyogenes*, anaerobic *streptococcus fæcalis hæmolyticus* and a pseudo-catarrhalis diplococcus. Therapeutic immunization with an autogenous compound vaccine of the usual strength in each element at first aggravated the whole malady without producing even intervals of improvement. I suggested to his doctor that the patient was hypersensitive to the pneumococcus element in the vaccine, and recommended that the dose of this should be reduced to one-tenth. This advice was acted upon, and the patient afterwards did extremely well, making a complete and lasting recovery.

A lady had suffered for many years from frequent attacks of bronchial asthma. The sputum yielded profuse growths of *streptococcus pyogenes* and *micrococcus catarrhalis*, both of which grew equally well under anaerobic and aerobic conditions. Therapeutic doses of autogenous vaccine were regularly followed by distinct attacks of asthma. Steady improvement took place, and the patient made an excellent recovery.

15. **Phthisis.**—It is in this disease that treatment by vaccines has probably been most extensively and generally used, but it has been too much confined to the employment of various tuberculins. The importance of investigating and treating the secondary infections of the bronchi and lung tissue has been little understood and rarely advocated, except by Allen (1, 2, 3, 36). He considers that the associated infections should be treated first, and that, when they have been brought under control, tuberculin treatment should be begun. As there is, however, no incompatibility of tuberculin with other vaccines, it seems to me to be unnecessary to delay the direct attack upon the tubercle

bacillus in these cases. Of the importance and value of fighting the associated infections I have seen convincing evidence. In view of the present lack of bacteriological laboratories, I admit that it is next to impossible for the practitioner to follow out this principle of treatment. There is, however, no valid reason why it should not be practised in sanatoria for phthisical patients.

The treatment of tubercle bacillus infections by tuberculin has already been dealt with in Chapter X., section 9. The treatment of secondary or associated infections in cases of phthisis is simply that of chronic bronchitis. Success need not be looked for in every case. There are some patients who seem to have no power of response to stimulation by tuberculin and in whom the bacillus pursues its course in spite of any measure that science has devised. In the large majority of cases it is, however, happily different, and there is no form of chronic infection in which, with the exception indicated, good results may be expected with more confidence from accurate therapeutic immunization than in pulmonary tuberculosis.

2. Diseases of the Alimentary Tract

1. Pyorrhœa Alveolaris, Gingivitis and Dental Caries.— The gums share with the nasopharynx the distinction of being the mucous membranes most prone to break down in their defences against common pathogenic bacteria. They suffer especially from various forms of chronic infection, generally commencing at the periodontal sulcus and tending to extend into the alveolus. The formation of alveolar pockets discharging pus, inflammation and spongy swelling of the adjacent soft tissues, and loss of teeth are the usual consequences. The infection may spread along the whole extent of the root of a tooth. It has, however, been demonstrated that deep infection of the alveoli may occur without either gingivitis or pyorrhœa. In several cases, I have examined extracted teeth that there had been reason to believe were the seat of deep infection, but which showed no pyorrhœa, and I have obtained the clearest evidence that the root was infected, in most of the cases, by an anaerobic

streptococcus pyogenes, accompanied by anaerobic *micrococcus catarrhalis*. This is a form of obscure infection that is often of great importance as a cause of chronic rheumatism. It is to be remarked also that severe forms of gingivitis may occasionally be observed without pyorrhœa.

Much that is, I believe, quite erroneous has been written about the bacteriology of pyorrhœa alveolaris as the result of work with inadequate technique. Those who have not applied anaerobic methods to the investigation of a series of cases can never have attained accurate knowledge of the infective causes of this disorder.

Exactly how much importance ought to be attached to infection by spirochætes in pyorrhœa alveolaris seems still to be in doubt. A film of the pus stained by Fontana's method generally shows these micro-organisms in large numbers. As far as is definitely known, these are just the spirochætes that are saprophytic in every mouth. A claim has, however, been made by Kolle (see *Brit. Med. Journ.*, 25th Aug. 1917, p. 265) that he has identified a special spirochæte which, he thinks, plays the principal part in the causation of pyorrhœa. Nevertheless the fact remains that nearly every case can be cured in the early stages by therapeutic immunization against all the pathogenic bacteria, aerobic and anaerobic, that can be isolated from the seat of the lesion, without dealing with the spirochætes that are always present in the exudation. Some allege that they have similarly effected a cure by destroying the spirochætes. My experience would lead me to recommend accurate therapeutic immunization against the pathogenic bacteria ascertained to be present, and the use of local measures for the destruction of spirochætes. For this purpose every dentist has his own favourite method; the free use of peroxide of hydrogen as a mouth-wash is effective in most cases. It should be understood that the important bacteria are not merely lying in the pus, but are in the inflamed tissues, into which they often penetrate to a remarkable depth. It is therefore impossible to reach them by means of antiseptics of a strength that can be safely used.

All the generalisations that have been made about such and such a bacterium being the true cause are erroneous.

The flora varies from case to case, just as in chronic post-nasal catarrh and in chronic bronchitis. It is generally more extensive than has been supposed, for it commonly includes anaerobic types that are not revealed by the methods usually employed. Much error has also been introduced into the literature of the subject by faulty analysis of the streptococcus flora, a point in regard to which even recent literature is lamentably defective. *Streptococcus pyogenes*, regarded by so many as the essential cause of pyorrhœa, may be absent. Two forms of chronic infection that are very common have, I believe, passed almost unobserved—namely, by the bacillus of influenza (on account of the difficulty there is in growing it) and by an anaerobic *micrococcus catarrhalis* (because only aerobic methods have, as a rule, been applied).

I have investigated 50 cases of pyorrhœa alveolaris. The observations have extended over many years, and only in the latter 25 cases were anaerobic methods systematically applied. All the following figures referring to anaerobic bacteria might therefore probably be doubled. This explanation is important, for otherwise an erroneous impression would be given of the frequency of anaerobic infections. In 6 of the cases, *streptococcus pyogenes* could not be found. Nevertheless, a streptococcus having the characters of *pyogenes* was the pathogenic bacterium most frequently present; it occurred in 44 cases as an aerobe and in 5 of these also as an anaerobe. *Streptococcus anginosus* was found in 27, aerobic *micrococcus catarrhalis* in 25, anaerobic *micrococcus catarrhalis* in 11, the bacillus of influenza in 15, aerobic pneumococci in 12, anaerobic pneumococci in 2, aerobic diphtheroid bacilli in 10, and anaerobic diphtheroid bacilli in 1. A severe form of *bacillus proteus* infection occurred in 2. *Streptococcus salivarius* of the inulin fermenting type was found in 3, *streptococcus fæcalis hæmolyticus* in 2, and the Bordet-Gengou bacillus and *staphylococcus pyogenes albus* in 1 each.

Treatment by therapeutic immunization with autogenous vaccines is generally completely successful in cases that are not far advanced. In very advanced cases with deep infection of the alveoli, it is impossible to eradicate the bacteria,

which are apparently entrenched in such a way as to be secure from the attack of polymorphs and antibodies. It is, however, of great advantage to immunize the patient before extraction of the teeth. The harmful consequences of sepsis of the lacerated tissues can thereby be completely avoided in most instances. In cases of pyorrhœa alveolaris, with gums that bleed extremely readily, it is important to investigate the possibility of there being a scorbutic element in the malady. I have seen at least one case in which this was found to be a complication, and in which benefit quickly resulted from antiscorbutic measures, when therapeutic immunization had failed.

The possible consequences of pyorrhœa alveolaris and deep alveolar infection, in addition to loss of the teeth, are especially anæmia, rheumatism, rheumatoid arthritis, chronic gastric catarrh and gastric and duodenal ulcer. Pathogenic bacteria may extend from the gums to the respiratory tract, but infections of the latter are common without pyorrhœa alveolaris.

It would be of considerable practical interest to know in what respects, if any, the pathogenic bacteria that produce ordinary dental caries differ from those that cause pyorrhœa alveolaris, but, so far as I know, anaerobic blood methods have not yet been applied to the problem. There are good grounds for believing that suppression of the bacteria that cause pyorrhœa results in a diminution, at least, of the tendency to localized dental caries. In all probability the infections do not materially differ in the two maladies, and localized dental caries simply represents the consequence of a wound infection of the teeth, analogous to the wound infections of trees.

Acute ulcerative gingivitis is an important, but not very common, malady that requires separate consideration. Claude G. Colyer (*Brit. Med. Journ.*, 12th Oct. 1918) has reported a series of cases in a paper of much practical value. He describes the disease as an acute inflammatory condition of the margins of the gums, which spreads rapidly and leads to sloughing of the interdental papillæ and of the gums around the necks of the teeth, ulceration of the adjacent mucous membrane and rarefying ostitis in chronic cases.

There is generally hæmorrhage from the gums, and much pain. He regards the disease as of the same origin as Vincent's angina, and dependent upon infection by *bacillus fusiformis* and spirilla. Therapeutic immunization does not seem to have been applied. Colyer recommends especially the swabbing of the gums with a mixture of vinum ipecacuanhæ, liquor arsenicalis and glycerine.

2. **Stomatitis and Glossitis.**—The only form of these maladies that I wish to deal with here is one, probably by no means rare, due to chronic infection of the tissues by a pneumococcus. I have seen two examples. I had an opportunity of treating only one of the cases, the other being a patient in a distant asylum. The first case was that of a naval officer who for several months had suffered from frequently recurring attacks of severe inflammation, occurring simultaneously in several patches on the mucous membrane of the mouth and on the tongue. Bacteriological investigation showed that these patches contained pneumococci, either alone or accompanied by *streptococcus anginosus*. I have no doubt that the pneumococcus was the leading infection. Under therapeutic immunization, the malady was completely arrested.

3. **Acute Tonsillitis.**—The chief bacterial causes of acute tonsillitis are virulent strains of *streptococcus pyogenes*, *streptococcus anginosus*, *pneumococci*, *staphylococcus pyogenes*, *bacillus influenzæ* and *bacillus diphtheriæ*. There is also to be added the fusiform bacillus which has been found occasionally to cause epidemics. Treatment by local measures, and by anti-serum in cases of acute diphtheria, is generally regarded as sufficient, but there is no reason why therapeutic immunization should not be used as in other acute infections. I have myself applied it with apparent success in quinsy due to *streptococcus pyogenes*. The requirements of the great majority of cases would be met by a polyvalent vaccine containing strains of *streptococcus pyogenes* from acute lesions, *streptococcus anginosus* and *staphylococcus pyogenes albus* and *aureus*.

4. **Chronic Tonsillitis.**—The most important bacterial causes are *streptococcus pyogenes, streptococcus anginosus, staphylococcus pyogenes,* pneumococci, *micrococcus catarrhalis,* *bacillus*

influenzæ and diptheroid bacilli. Most cases are eminently suitable for treatment by therapeutic immunization.

5. **Gastric Catarrhs.**—In my opinion there is satisfactory evidence that many forms of acute and chronic catarrh of the stomach are essentially due to extension of infections from the oral or nasopharyngeal regions to the gastric mucosa. I have observed several cases in which doses of *streptococcus pyogenes* or of *streptococcus anginosus*, isolated from the gums or nasopharynx, have been followed by distinct reactions in the stomach in persons who were suffering from chronic dyspepsia. Many patients after immunization against bacteria infecting the gums or the respiratory tract spontaneously report that they are digesting their food better.

I believe that therapeutic immunization has a useful application in many cases of gastric disorder. As infections of the stomach are almost always the result of the extension of an infection from above, one can rarely be wrong in using as a vaccine any pathogenic streptococci that can be isolated from the mouth or nasopharynx, or from the sputum.

Hale White and Eyre (18) have recorded a case of gastric catarrh in which *bacillus coli communis* was found in the stomach; under therapeutic immunization against this bacillus the patient recovered.

6. **Gastric and Duodenal Ulcers.**—The suggestion of the application of therapeutic immunization to such lesions at the present day is likely to be received with derision. Nevertheless, it has been sufficiently shown by experience that in this class of cases therapeutic immunization is destined to achieve some of its greatest triumphs. Hale White and Eyre (18) have recorded two cases in which *streptococcus pyogenes* was isolated from abscesses that developed in connection with gastric ulcers; both patients recovered under surgical measures and therapeutic immunization against this streptococcus.

I have investigated three cases of duodenal ulcer by examination of the flora of the stools. In each case this included very abundant colonies of *streptococcus pyogenes*, in one of the cases the proportion being about 400 to 1 coliform bacillus colony. *Streptococcus pyogenes* does not occur normally in the stools; it is present occasionally in cases in

which there is no evidence of the existence of gastric or duodenal ulcer, but the patients are always out of health. In each of the three cases of duodenal ulcer treated by autogenous *streptococcus pyogenes* vaccine, the patients experienced focal reactions in the lesion, and continued therapeutic immunization was followed by more or less complete restoration of the patient to health. I have had no opportunities of studying other cases, but I think the evidence of these three is sufficient to warrant the recommendation that in cases of gastric and duodenal ulcer the flora of the stools should be investigated, and that if *streptococcus pyogenes* is present a course of therapeutic immunization should be tried. The initial dose should be small, as a severe focal reaction in the ulcer might cause hæmorrhage.

7. **Acute Infective Disorders of the Intestine.**—The application of therapeutic immunization to typhoid and paratyphoid fevers and to dysentery has already been considered under the specific bacilli in Chapter X. Among the other bacterial causes of acute enteritis are pneumococci, *streptococcus pyogenes*, *bacillus proteus*, *bacillus pyocyaneus* and Morgan's bacillus.

I have seen several examples of *bacillus proteus* infection of the intestine, though most of them tended to produce a chronic, rather than acute disorder.

In these, as in other acute infective disorders, therapeutic immunization has its legitimate application.

8. **Chronic Infective Disorders of the Intestine.** — The bacterial causes of chronic intestinal disorders are very numerous, and some of them, though of great importance in practical medicine, are as yet little understood. This remark applies especially to chronic infections by pneumococci, *streptococcus pyogenes*, *streptococcus fæcalis hæmolyticus*, anaerobic diphtheroid bacilli and anaerobic streptothrices.

It is clearly established that *bacillus coli communis*, although a normal inhabitant of the intestine, frequently assumes a pathogenic action in the alimentary tract. Hale White and Eyre (18) have recorded three cases of this kind which recovered under therapeutic immunization. A common type of case is that in which *bacillus coli communis* attacks the cæcum, or ascending colon, and, either through the

lymphatics or blood-stream, reaches the urinary tract, where it sets up acute cystitis. It is strange how, in such cases, attention is almost always directed exclusively to the bladder, whereas it is just as important to treat the intestinal lesion.

Chronic pneumococcus infections of the intestine are, in my experience, fairly common. For some years, I have repeatedly drawn attention to them in published papers, but those who are in a position to guide medical opinion will have nothing to do with the matter. Two types may be distinguished—namely, an infection by a pneumococcus of ordinary characters and one by a pneumococcus specially characterized by the intensity of its hæmolytic or, more correctly, hæmoglobinolytic, action. It cannot, however, be said that there is any hard and fast line between the two types. Only the first need be considered here ; the second will be more properly dealt with in connection with the subject of therapeutic immunization in pernicious anæmia.

I have observed 32 cases of chronic infection of the intestinal tract by pneumococci in persons who had not the signs of pernicious anæmia in their blood. In a small proportion of these the pneumococcus was intensely hæmolytic, as in cases of this disease. In many of the cases the pneumococcus infection was complicated by others, but the characteristic symptoms can be deduced from the several cases in which no other important infection was observable. The patients are obviously out of health. They complain that they never feel well. They have no energy for work, and are generally depressed. They are anæmic and thin. Many are distinctly neurasthenic, some severely so. Most of them have occasional attacks of diarrhœa, and a varying amount of pain and discomfort about the abdomen. The following are some illustrative cases :—

A gentleman had suffered for twelve years from recurrent attacks of severe pain in the abdomen, chiefly on the right side, and lasting from three to four days. I found that the intestinal flora showed about two colonies of pneumococci to one of *bacillus coli communis*. An autogenous vaccine produced focal reactions in the intestine, and after a course of immunization the attacks ceased.

An army officer who had been anæmic and out of health for several years, and who had well-marked symptoms of neurasthenia, was found to have an intestinal flora showing about 100 small colonies to

one coliform bacillus colony. The small colonies consisted chiefly of pneumococci, but also of *streptococcus fæcalis hæmolyticus*. Under therapeutic immunization the patient was completely restored to health, although not until a second course of immunization had been given, a year after the first.

An army officer had suffered for two or three years from chronic diarrhœa. The initial attack resembled dysentery, but it was ascertained that his trouble was not of this nature. I found that he had evidence of a severe intestinal infection by a pneumococcus. Under therapeutic immunization he had a complete recovery.

Chronic intestinal infections by *streptococcus pyogenes* are also common, though not so frequently to be observed, in my experience, as pneumococcus infections. I have seen sixteen cases. Several hundred controls, compared with these cases, support the conclusion that this streptococcus is never present in the intestine, except as an important pathogenic agent. In three of the sixteen cases the patient suffered from gastric or duodenal ulcer. Nearly all of the remaining cases were complicated by anaerobic diphtheroid bacillus infection, and were severely neurasthenic. Many suffered from intense abdominal pain ; some had recurrent attacks of diarrhœa. The following is a typical case :—

A gentleman of middle age had suffered for about ten years from attacks of acute pain in the region of the ascending colon, generally lasting for about three days. They were, he stated, accompanied by slight rise of temperature, splitting headache, acute indigestion and total unfitness for exertion. I found that his stools contained, in large numbers, two distinct types of *streptococcus pyogenes*, one of which grew only aerobically and the other only anaerobically. Under therapeutic immunization he experienced distinct focal reactions, ceased to have attacks, and gained weight. A supplementary bacteriological examination, made after the course was finished, showed that *streptococcus pyogenes* had disappeared. There remained a very high proportion of colonies of *streptococcus fæcalis hæmolyticus* and of anaerobic diphtheroid bacilli, indicating corresponding infections, for which further therapeutic immunization was advised.

Colonies of *staphylococcus pyogenes* may occasionally be found to be conspicuous elements in an intestinal flora. I have observed seven cases of this nature. All were those of patients who were suffering from more serious intestinal infections sufficient to account for the symptoms, and it was

therefore impossible to estimate the part played by the staphylococcus.

The bacillus of Friedländer is from time to time found to be the sole coliform bacillus in the intestine; more commonly it is one of the constituents of the coliform flora. In many cases this bacillus may certainly be as innocent of pathogenic action as *bacillus coli communis* in the healthy person. I have observed only three or four cases in which it appeared to be exercising a pathogenic action. For example, in one case it seemed to be the cause of a severe proctitis.

Bacillus proteus is also an occasional constituent of the intestinal flora. In four or five cases that I have observed, its pathogenic action has been pretty clearly established. In these cases its colonies predominated in the flora, and diarrhœa and general toxic symptoms were prominent features.

Chronic intestinal infections by anaerobic diphtheroid bacilli have already been considered in the preceding chapter, section 7. Chronic infections by anaerobic streptothrices have likewise been dealt with in Chapter X., section 14.

9. **Mucous Colitis.**—The discharge of mucus with the stools is a common occurrence in various forms of acute and chronic intestinal catarrh, and many such cases are often thought to be examples of mucous colitis. This term should be reserved for a special type of case with sufficiently characteristic symptoms. The malady is one that certainly occurs chiefly in women. Most writers on the subject mention cases occurring in men, but there are strong reasons for doubting if these were genuine examples of the disease. If true mucous colitis does occur in the male, it must have a pathogenesis, analogous to that now, I believe, clearly established for the disease in the female subject.

I have investigated six cases, all occurring in women. The special feature of the malady consists in the discharge of large quantities of tenacious mucus from the bowel, continuously, or in association with special attacks, and accompanied by severe abdominal pain and nervous prostration. There is in nearly all cases obstinate constipation from which relief is obtained only by washing out of the bowel. The

patients are always thin and neurasthenic, and though their appetite is good they are afraid to eat, lest they should unwittingly take something that will disagree with them and precipitate an attack. The malady seems to be much more common on the Continent than in this country. Its pathology has been discussed by many observers, but none of them, as far as I have been able to ascertain, has grasped the true significance of the symptoms, and treatment remains merely palliative and extremely unsatisfactory.

The facts observed in the cases that I have investigated and treated warrant, I think, certain important conclusions that define the true nature of the malady and point clearly to methods of successful treatment, although I freely recognize that there is still much to be investigated regarding the infections that occur and the nervous reflex actions that are concerned in the intestinal disturbance.

All of the cases that I have investigated have suffered from active infections of the uterine appendages, in some complicated by backward displacement of the uterus. These infections are of the kind indicated in the section on Diseases of the Genito-Urinary Tract (section 3), and need not be recapitulated here. In the intestinal tract there is always a severe infection by anaerobic diphtheroid bacilli, and generally also by other important bacteria, as, for example, by *streptococcus fæcalis hæmolyticus*, bacillus of Friedländer, *bacillus proteus*, and probably often by *bacillus coli communis* Not uncommonly there is enteroptosis of a severe kind and, frequently also, floating kidneys. These undoubtedly are often aggravating features, but a study of a series of cases shows that they are not essential causes of the malady. There are typical cases of mucous colitis in which there is no enteroptosis. Ernest Henry Harrison (*The Lancet*, 21st Sept. 1907) found that only 12% of his cases had this complication. This observer noted that the malady was usually preceded by infections of the uterus and appendages.

The essential causes are reflex irritation from the Fallopian tubes and ovaries, and a peculiar irritability of the mucous membrane of the colon, determined by an anaerobic diphtheroid bacillus infection. An attack is precipitated

generally by one or other of various causes that increase the bacterial toxic action, and by congestion in the uterine appendages. The hypersensitiveness of the colon may be increased by an exacerbation of the existing infections, or the onset of new secondary infections. I have known this to occur as the result of an accidentally acquired intestinal infection by *bacillus proteus*.

In applying therapeutic immunization to the treatment of disease, we are ever endeavouring to eradicate primary causes. In mucous colitis we have to investigate and suppress existing infections of the Fallopian tubes. Repeated bacteriological examinations of menstrual blood swabs will generally reveal an important infection. This must be treated by means of autogenous vaccines, and the immunization should not be stopped until bacteriological examination has shown that the infection has been eradicated. Similarly, all existing intestinal infections must be treated by therapeutic immunization. If, after the application of such measures, symptoms continue, it may be safely inferred that there is a uterine displacement, or a gross lesion of the uterus or its appendages that can be properly dealt with only by surgical measures.

10. **Appendicitis.** — The treatment of appendicitis is generally regarded as lying purely within the domain of the surgeon, but since it is essentially due to local bacterial attack it must equally fall within that of the bacteriologist. *Bacillus coli communis*, various species of *streptococcus*, pneumococci, *staphylococcus pyogenes*, and *bacillus pyocyaneus* are the bacteria that have been most commonly found in the inflamed tissues. Why these bacteria should attack the region of the appendix is a problem very similar to that which we meet with in regard to local attack upon the tonsils and duodenum. Some special factors exist, or are apt to arise, that place these regions at a disadvantage in their resistance to common pathogenic bacteria that are more or less constantly present in small numbers in the alimentary tract.

In chronic cases, or in patients who it is thought are threatened by an acute attack, it may be laid down that the systematic investigation of the bacterial flora of the stools

will in most cases reveal the presence of an excessive number of certain pathogenic bacteria that throws light upon the infective cause at work in the cæcum. Immunization against these must place the patient in a better position, and may in many instances obviate the necessity for operation. If local infection by *bacillus coli communis* is suspected, an autogenous vaccine should be prepared and administered in an initial dose of about 0·02 mg. If there is an infective focus in the intestine, a focal reaction is certain to occur, indicated by local pain and tenderness, in from twelve to twenty-four hours after the dose has been given. If such a reaction is obtained, a progressive course of immunizing doses should be administered.

Therapeutic immunization has also an important but still unrecognized field as a prophylactic measure prior to operation in cases in which this procedure is necessary. In many cases there is time before operation to have an analysis made of the intestinal flora and to immunize the patient against pathogenic bacteria found to be present. On account of the frequency of attack by *bacillus coli communis*, probably this bacillus should be included in the immunization in all such cases.

11. **Chronic Intestinal Stasis.** — The causes, pathology, consequences and treatment of this extremely common malady have been widely discussed in recent years. The views most in favour are those of Arbuthnot Lane, who has boldly applied operative measures for the relief of some of the more severe cases. As is well known, his main contention is that unsuitable diet in childhood and assumption of the erect posture result in delay of the fæcal material in the large bowel, and that, in consequence, new membranes form in the peritoneum as the result of "crystallization of lines of force," with the object of resisting downward displacement. These bands in some cases become a source of danger to the patient, and may tend to aggravate the intestinal stasis. These views have been criticized by Adami (*Brit. Med. Journ.*, 1914, i., p. 177), who maintains that in cases of intestinal stasis the important element is a subinfection, or a low form of infection of the alimentary tract.

Arbuthnot Lane attributes almost every kind of bodily

disorder to intestinal stasis. It is strange that most of them occur quite commonly in persons who do not suffer at all from intestinal stasis. The view generally held is that an auto-intoxication results from absorption of disintegration products of the food material too long retained in the canal. Chief importance is attached to the toxic action of amines, and especially to parahydroxylphenylethylamine, which has an action similar to that of adrenalin. It is decomposed in the liver, and may be detected in the urine in its altered form. It raises the blood pressure and is alleged to give rise to arteriosclerosis, interstitial nephritis, rheumatoid arthritis, exophthalmic goitre and mucous colitis. Neurasthenia is excluded, probably because it is thought correct at the present day to regard this malady as purely of psychic origin.

There is certainly much truth in these views, but they err, I believe, by neglect of the bacterial factors that are at work. A little reflection should be sufficient to convince anyone with even an elementary knowledge of bacteriology that bacterial factors there must be, and yet the orthodox medical opinion of the present day is quite satisfied with the dietetic and mechanical theories of the origin of intestinal stasis, and with the purely chemical explanation of its harmful consequences. It is much to be regretted that Adami's views have not influenced medical opinion and pathological research, as they are entitled to have done.

The question of the possible bacterial origin of chronic intestinal stasis ought to be made the subject of systematic investigation in suitable cases occurring in the wards of a children's hospital. For my own part, I have had opportunities of studying the intestinal flora only in established cases in the adult. In over 90% of cases the patients are suffering from anaerobic diphtheroid bacillus infection, and they generally have at the same time other important abnormalities of the intestinal flora. In cases in which there is no anaerobic diphtheroid bacillus infection there is almost always a well-marked aerobic diphtheroid bacillus infection. These two types of infection may be combined. It is easy to allege that these infections are consequences and not causes of the intestinal stasis, but where is the proof?

The available evidence tends very strongly to support the opposite view. If anaerobic diphtheroid bacilli do not cause intestinal stasis, they certainly produce very important nervous symptoms, for, in scores of cases, these symptoms have been relieved by therapeutic immunization. Moreover, in many cases, it has been observed that overdoses of the vaccine caused aggravation of the characteristic nervous symptoms. Therefore these anaerobic diphtheroid bacilli are neurotoxic organisms. Now, a very natural action of a neurotoxic bacillus in the colon must be to injure the nervous mechanism upon which peristalsis depends. This is, I maintain, exactly what has occurred. The patients have, often at an early age, begun to suffer from a chronic infection of the bowel by these neurotoxic bacteria, with the consequence that peristaltic action became more or less paralysed. The complete proof of this view must lie with the results of the investigation of a series of early cases. Successful therapeutic immunization against these anaerobic diphtheroids does not relieve the constipation, but this may be explained by the fact that the damage to the nervous mechanism upon which peristalsis depends is of an irremediable nature. To cure intestinal stasis by therapeutic immunization it would be necessary to treat the patient within a few months of the onset of the infection. It will some day probably be shown that this can actually be done.

The action of toxic products developed in the food residues and secretions too long retained in the colon, and especially of amines, is no doubt extremely important. There are patients suffering from chronic intestinal stasis who state that if they allow more than twenty-four hours to pass without relieving the bowel by enemata they suffer from lassitude, mental depression and confusion, amnesia, irritability and headache. But important as the toxic action of these disintegration products may be, there is no reason why the action of bacteria actually infecting the mucosa should be left out of consideration. That anaerobic diphtheroid bacilli do invade the mucosa has been proved by histological examination of the intestinal wall by myself in a long series of cases, and by the common appearance of the same bacilli in the urine, which they can have reached only through the

blood-stream or lymphatics. Especially in long-standing cases, the addition of other infections is the rule. *Streptococcus fæcalis hæmolyticus*, pneumococci and *streptococcus pyogencs* are important in this connection, but *bacillus coli communis* is probably one of the most frequent invaders, although evidence of its attack is not always easy to obtain. Its pathogenic action in the intestine is certainly much commoner than the occurrence of *bacillus coli* cystitis, and this, which is a consequence of it, is common enough.

If these views are in accord with fact, the indications for treatment are clear. While intestinal stasis is relieved by the use of measures applicable to the case, a subject that does not call for consideration in this book, we may, in many patients who suffer from evidences of bacterial invasion, do much to relieve suffering by a course of therapeutic immunization based upon an accurate bacteriological investigation of the stools. This is especially true in the case of those who suffer from severe neurasthenic symptoms.

3. Diseases of the Genito-Urinary Tract

1. **Cystitis and Pyelitis.**—Bacteria may pass through the urinary tract without attacking its walls. Various pathogenic diphtheroid bacilli commonly do so, but also, comparatively rarely, many other species, including bacilli of the coliform group and streptococci. Infection of the mucosa is always indicated by the occurrence of large numbers of polymorphs in the urine. Any pathogenic bacterium reaching the urinary tract by way of the blood-stream or lymphatics may set up pyelitis or cystitis. Exposure to cold and fatigue may lay the subject open to attack and determine, for example, the onset of acute cystitis due to infection by *bacillus coli communis*. It is believed that bacterial infection of the bladder rarely occurs without involvement also of the pelvis of the kidney.

The infective causes of cystitis and pyelitis are numerous. They will perhaps be most clearly understood from the results of examinations made in a fairly large series of cases in which a diagnosis of cystitis could be made from the presence of abundant polymorphs in the urine and the

symptoms complained of by the patient. I have records of bacteriological investigations in 50 cases (acute and chronic). *Bacillus coli communis* was the infective cause in 25, the bacillus of Friedländer in 9, *streptococcus fæcalis* in 5, *streptococcus fæcalis hæmolyticus* in 3, *bacillus proteus* in 3, the tubercle bacillus in 3, aerobic diphtheroid bacilli in 5, anaerobic diphtheroid bacilli in 2, *bacillus lactis acrogenes* in 1, *bacillus cloacæ* in 1, and *staphylococcus pyogenes aureus* in 1. In six of the cases there was a double infection, and in one a triple infection.

Several cases of cystitis in persons suffering from advanced tabes dorsalis have been excluded from these statistics, which represent an ordinary set of cases in this country, such as any practitioner might be called upon to treat. Among the tabetics, one additional bacterial cause of chronic cystitis was observed—namely *diplococcus crassus*—which occurred in two distinct forms. One of the points most worthy of notice in the above series of 50 cases is the frequency of infections of the bladder by the bacillus of Friedländer. All of the cases with a diphtheroid cystitis had prominent nervous symptoms. Two had spastic paralysis; one had weakness of the legs and symptoms that suggested to her doctor that she had disseminated sclerosis; another had what was described as "neurasthenia and bladder irritation"; and a man whose urine was loaded with aerobic diphtheroids suffered chiefly on account of almost complete paralysis of the sphincter of the bladder. The three cases of tuberculosis showed pure infections. Two of them were treated with autogenous vaccines and did well, the bacilli in the urine being reduced in number almost to vanishing point. Both patients were advanced in years and died from intercurrent illnesses.

Most cases of acute and subacute cystitis respond well to therapeutic immunization, if autogenous vaccines are used in accordance with the principles already laid down. Chronic cases are less favourable as regards the prospects of complete cure, but even when it is impossible to eradicate the infection entirely, it is generally possible to restore the patient to health by a course of vaccines, and to prevent recurrence of symptoms by continuing to give a fortnightly dose

sufficient to cause focal and general reactions of moderate intensity.

2. **Urethritis and its Complications.**—The lower end of the male urethra normally harbours some bacteria, which may include diphtheroid bacilli, staphylococci, *streptococcus fæcalis*, *diplococcus crassus* and *bacillus coli communis*. We need not be misled by the occurrence of these saprophytes. A consideration of the symptoms and the study of a carefully secured specimen of discharge will generally enable us to determine accurately if there are any active infections. The chief bacterial infective factors in acute and chronic urethritis are the gonococcus, *staphylococcus pyogenes*, *bacillus coli communis*, *streptococcus pyogenes* and various species of diphtheroid bacillus.

The treatment of acute urethritis of gonococcus origin by therapeutic immunization has already been considered in Chapter X., section 5.

Epididymitis, vesiculitis and prostatitis are generally bacterial inflammations. Aerobic and anaerobic gonococci, diphtheroid bacilli and staphylococci are probably the most common causes. It would, I think, mark an advance if surgeons made it a practice to obtain a bacteriological report on the conditions in cases especially of prostatic enlargement, and carried out a short course of immunization against any ascertained infection before operating. Probably fewer cases would then go wrong.

A bacteriological study of thirty cases of chronic urethritis has brought out some interesting points. Anaerobic gonococcus infections appear to be common as the essential cause, and there can be no doubt that they have in the past escaped detection. Seven of the cases were of this nature, whilst five showed aerobic gonococcus infections. The series included several cases of severe chronic urethritis due to the action of aerobic and anaerobic diphtheroid bacilli, as was confirmed by the results of therapeutic immunization. Other causes were found to be *staphylococcus pyogenes*, *streptococcus pyogenes* and staphylococci of the mannite non-fermenting group.

3. **Puerperal Infections.**—Many cases of puerperal infection are septicæmias in which the uterus is the infective focus. *Streptococcus pyogenes*, *pneumococci*, *staphylococcus pyogenes*

albus and *aureus*, aerobic and anaerobic diphtheroid bacilli and *bacillus coli communis* are among the most important infective agents, the first being the essential one in the large majority of cases. Anti-streptococcus serum has been used with a considerable measure of success.

Henry A. Craig (25) states that in his experience therapeutic immunization has been of great value in cases of this kind. Wynn (31) has also recorded numerous cases treated successfully by vaccines. Acute infections of this nature should, on general grounds, be capable of suppression, in most instances, by specific stimulation of the patient's defences. Rapid methods of bacteriological diagnosis and of preparing autogenous vaccines (requiring less than twenty-four hours) are urgently needed for such cases. The difficulties are not insuperable.

The principles to be followed are exactly those already indicated as applicable to acute infections in general. It is taken for granted that local therapeutic measures will be employed at the same time.

4. **Other Infective Diseases of the Female Genital Tract.**—*Leucorrhœa* is always associated with local bacterial infection, although the influence of uterine displacement must be taken into account in some cases. The various common infections include those by gonococci, *staphylococcus pyogenes*, staphylococci of the mannite non-fermenting group, various species of diphtheroid bacillus, *bacillus coli communis*, *streptococcus pyogenes*, and *bacillus proteus*. Accurate therapeutic immunization, if combined with suitable local measures, is clearly applicable.

Acute and chronic forms of Endometritis, due to extension of the action of pathogenic bacteria of the kind already named, are apparently at the present day rarely made the subject of bacteriological investigation, and still more rarely treated by therapeutic immunization. Nevertheless, they must constitute an excellent field for the successful employment of this measure. Inaccurate bacteriological diagnosis, based upon the examination of only aerobic cultures, is probably responsible for many discouraging experiences in cases in which vaccines have been tried. Many of the infections are essentially anaerobic ones.

I have investigated 22 cases of endometritis in which a swab taken from the interior of the uterus was supplied by the attending doctor, or by a specialist to whom the patient had been sent, and the results are, I think, instructive. By far the most frequent bacterial cause (confirmed in most of the cases by the success of therapeutic immunization) was one or more species of diphtheroid bacillus. Aerobic types occurred as pure infections in 5 cases and an anaerobic type as a pure infection in 1. As a rule, however, these aerobic and anaerobic diphtheroid bacillus infections were combined, or they were associated with other infections. Altogether, 12 of the 22 cases had severe aerobic diphtheroid bacillus infections, and 9 had anaerobic diphtheroid bacillus infections. Mannite non-fermenting staphylococci were abundant in 7 of the cases, aerobic *streptococcus pyogenes* in 5, and anaerobic gonococcus in 2. The other pathogenic species obtained were *streptococcus fæcalis*, *streptococcus fæcalis hæmolyticus* (aerobic in one case and anaerobic in another), pneumococci and an anaerobic *streptococcus pyogenes*.

Specially instructive is the case of a married woman who suffered from severe mucous colitis and pelvic disturbances. Aerobic cultures on hæmoglobin media yielded nothing, and therefore, with the methods of examination commonly employed, it would probably have been judged that there were no uterine infections. Anaerobic cultures on the same media yielded, however, profuse growths of gonococci, diphtheroid bacilli and *streptococcus pyogenes*.

Salpingitis and ovaritis are likewise generally of bacterial origin. The difficulties of investigation are great, but there is one method by which these can frequently be surmounted. This is by the bacteriological examination of a menstrual blood swab. Infection of the tubes always occurs as an extension from the uterus, and it has been clearly established that a knowledge of the flora of the menstrual blood, taken in conjunction with the occurrence of symptoms of inflammation of the Fallopian tubes, often warrants a diagnosis of the nature of the most important infections upon which these symptoms depend. I have investigated 19 cases by this method. In at least 14 of these the examinations

were made because of pelvic symptoms that indicated the occurrence of salpingitis. In 12 of these cases, anaerobic cultures on +6 hæmoglobin agar revealed the presence of gonococci. In 5 of these cases the patient suffered from a painful form of rheumatoid arthritis, and in the remaining 7 from severe neurasthenia. In 2 of the cases of this kind in which therapeutic immunization was carried out by myself, focal reactions and ultimate improvement confirmed the importance of the infection. In this important class of infection, at least, the study of the bacterial flora of a menstrual blood swab is therefore of great value. Other pathogenic bacteria are commonly found, but to what extent they are infecting agents of the uterus and tubes this method of investigation does not always clearly reveal. Whether they should be included in the immunization or not must rest with the judgment of the observer. It may often be of advantage to include some of them, especially anaerobic diphtheroid bacilli, *streptococcus pyogenes* and *staphylococcus pyogenes* when they are present in profusion.

5. **Tuberculosis of the Urinary Tract.**—Cases of tuberculosis of the urinary tract, if obtained early enough, seem to be specially suitable for treatment by vaccines. When the tubercle bacillus is present alone in the urine, an autogenous vaccine may be prepared without much difficulty, and its use has distinct advantages over tuberculin. In a case of infection of the prostate in a man aged fifty-two, I used autogenous tubercle bacillus vaccines with considerable success. The dose that suited best was one of about 0·00005 mg. It was followed after three days by a distinct focal reaction, which occasioned increase of local discomfort, frequency of micturition and the appearance of a little blood in the urine. The tubercle bacilli diminished in numbers, until they almost disappeared, many of the distressing symptoms were relieved, and the patient gained weight. He died from an intercurrent malady some time after treatment had been stopped.

4. Diseases of the Nervous System

1. **Neuralgias and Neuritis.**—These maladies assume very many forms, and they may be due to many different causes.

A large proportion of them are essentially dependent upon infective processes and their associated toxic actions. The common infections, conveniently referred to as "rheumatic," are responsible for very many cases. In my experience, *streptococcus pyogenes*, *streptococcus anginosus* and pneumococci have been found in numerous instances to be present as the agents of a severe infection of the gums, or nasopharynx, and therapeutic immunization has resulted in recovery from the painful symptoms. Another common bacterial cause of neuralgias and neuritis is anaerobic diphtheroid bacillus infection of the intestine. It may occur in association with rheumatic affections of the joints, due primarily to other bacterial toxic actions, and, unless it is detected and treated, therapeutic immunization may fail to relieve the patient's sufferings. A special interest attaches to the possible infective causes of sciatica and lumbago. As a rule these maladies seem to be dependent upon *streptococcus pyogenes*, or *streptococcus anginosus* infections of the gums, fauces or nasopharynx; the intestinal flora may be normal, but it is certainly not always so. I have observed a case of sciatica in which, in addition to severe infection of the gums by *streptococcus pyogenes*, *streptococcus anginosus* and pneumococci there was a well-marked intestinal infection by the last-named bacterium.

Practical experience has shown that many cases of neuralgia and neuritis are benefited by *staphylococcus pyogenes* vaccines. The action must, I think, be regarded as a pharmacological one, as there is little evidence that would justify us in regarding this staphylococcus as a cause of these maladies. A stock vaccine that has been largely used for the treatment of this type of case is one of the following composition :—

1 c.c. =

Staphylococcus pyogenes aureus et albus	0·5	mg.
Streptococcus pyogenes (aerobic and anaerobic strains from cases of rheumatism)	0·1	mg.
Streptococcus anginosus	0·1	mg.
Pneumococcus (from cases of rheumatoid arthritis)	0·005	mg.

Dose—0·1 c.c. to 1 c.c.

In cases in which it is desired to try therapeutic immunization with autogenous vaccines, a thorough search should be made for chronic infective mischief in the gums, fauces, nasopharynx, nasal passages, bronchi, and intestinal tract. Occasionally an important infective element may be found in the urine, as in the case of a patient who suffered from rheumatoid arthritis, primarily of gonococcus origin, and whose urine was found to be loaded with *streptococcus pyogenes*.

2. **Neurasthenia.**—The distinctive signs and symptoms of neurasthenia are capable of fairly precise definition, and there need rarely be any doubt, or difference of opinion, as to whether a particular case is to be classed as of this nature or not. The chief symptoms are a constant feeling of fatigue, not relieved by rest, and the occurrence of various forms of hyperæsthesia, paræsthesia and localized pain. Two important physical signs constantly occur—exaggeration of the patellar reflex and tremor of the eyelids when the eyes are half closed. Added to these, there are, in greater or less degree, characteristic mental features which constitute the picture of psychasthenia — namely, incoercible ideas, obsessions and monophobias.

Predisposing to the occurrence of neurasthenia there is a particular type of constitution—the neurasthenic diathesis. Under the same adverse conditions some persons will develop neurasthenia, while others will not. Slight degrees of the malady are extremely common. Indeed, to have the signs of neurasthenia in its mild form is a distinction, for it is certain that it is chiefly the people who have a neurasthenic constitution who are the most brilliant, original, energetic and influential. It is they who do the intellectual work of the world.

It is important to recognize that neurasthenia may be a prelude of serious organic disease, such as general paralysis, dementia præcox, other forms of insanity, pernicious anæmia, rheumatoid arthritis and tuberculosis, the onset of which may obscure, but rarely obliterates, the characteristic features of the less serious malady.

With regard to the causation of neurasthenia, the evidence has hitherto been lacking in precision, or altogether defective. The only assigned cause having any definiteness is trau-

matism, and this, at most, accounts for only a small proportion of the cases. Nevertheless, this factor is of special interest at the present time. The trauma may be physical, or it may operate by vivid and painful mental impressions, as shock. I believe that the importance of this traumatic, factor has been much exaggerated, and that many of the morbid conditions almost universally attributed to it can be proved to be due to chronic bacterial infections, which have been aggravated by the physical and mental stress, and other conditions inimical to health, to which the soldier on active service is inevitably subjected.

In recent years, a school has arisen among medical men and extended far beyond the limits of the profession, which explains neurasthenia and all the psychoneuroses as of purely psychogenic origin. In brief, these maladies are regarded as resulting from the internal conflict and turmoil produced by suppressed complexes, which can be revealed, and can have their injurious influences at the same time destroyed, by psycho-analysis. The journals have been flooded with papers on the subject, which is evidently one which appeals strongly to a particular type of mentality, marked by facility of verbal expression, but not, it must be said, by any conspicuous ability to handle evidence and mould it into a scientific system.

This is not the place in which it would be appropriate to endeavour to deal fully with the errors into which, in the judgment of many, the psycho-analysts have fallen in their interpretation of normal and morbid mental phenomena. The scope of this book requires, however, that the bacterial toxic factors in the pathogenesis of neurasthenia should be considered in some detail, and in entering upon a consideration of this subject I would lodge a strong protest against the total neglect of these factors by the writers to whom I have referred. The matter has been brought to their notice ; they have done nothing to refute the evidence ; they have simply ignored it. Science demands that, in seeking to reach the truth regarding any of its problems, all the known factors shall be taken into consideration. This rule the psycho-analysts have failed to follow, remaining, as has been aptly said, shut within the labyrinth of their own hermeneutics.

Although, as I have just indicated, I do not intend to enter here into a detailed criticism of psycho-analysis, it would probably be a mistake if I dismissed the subject without stating the main grounds upon which I think it must sooner or later come to be regarded as a false and unscientific system. A few words on the subject are therefore necessary here.

Normal mental operations are dependent upon integrity of the association centres of the brain, and, in cases in which there is mental aberration, it is essential to look to the question of these centres having been damaged in their structure. This consideration is entirely neglected by the psycho-analysts, who are continually dealing with disorders of a kind that we know to be dependent upon impairment of the action of the association centres by toxins. The future progress of psychological medicine will not be along the lines laid down by the psycho-analysts, but along those of a truly physiological psychology and of a scientific pathology, which will apply to mental diseases, as to other diseases, the light of modern bacteriology so essential for the interpretation of both.

The fundamental error of the psycho-analysts consists, in my opinion, in their conception of what mind is. To them, consciousness is a thing that can be observed and studied in others, like the colour of their hair, or the rate of their pulse, whereas, in the nature of things, it is impossible for anyone to observe it, except in himself. This being so, some of the writings of these authors are distinctly compromising, as, for example, to give a mild instance, the description, in terms of psycho-analysis, recently given by an eminent alienist in this country of the conscious and subconscious experiences in drunkenness. This, however, is a minor matter. The fundamental error to which I wish to draw attention is the psycho-analyst's conception of mind as a thing, the component parts of which can be laid away in the pigeon-holes of the unconscious, or subconscious, and brought forth to the light of consciousness with various degrees of facility, at will by the owner, or reluctantly and triumphantly by the psycho-analyst. To the exponents of psycho-analysis, mind is a thing which no doubt they claim to be immaterial, but which

nevertheless they handle just as if it were material. Now, mind is not a thing in this sense at all; it is only a concomitant of a reaction in the psychical centres of the brain. This, I maintain, is the only view that consists with the evidence available at the present day, evidence which the psycho-analysts continually ignore. The human brain is probably the most complex mechanism in all nature, but still it is only a mechanism, never manifesting its activities except when set in motion. Some simple analogies help us to understand its operations, and at the same time to display the wrong point of view taken by the psycho-analysts. One of the best is perhaps that of the gramophone. This consists essentially of a revolving disc with a groove in which certain impressions have been made, and a sound box, the operating point of which is a needle that is adjustable to the groove in the disc. If the instrument is in good working order, when we place the needle in the groove and set the disc in motion, certain harmonious sounds are produced. If we regard the instrument as aware of these sounds, we have an exact analogy to what mind is. On the disc there may be impressed the exact record of one of the finest compositions, but these impressions are not music. So with the psychical centres of the brain. They contain records of extraordinary complexity, but they are merely impressions and not mind; only when stimuli reach them from other portions of the nervous system, and they react to these stimuli, does consciousness result. This consciousness, like the sound proceeding from the gramophone, is not a thing lodged in the instrument, but merely a reaction that can be produced in the instrument by appropriate stimulation. Mind, like sound, is to be regarded as a form of motion. The error of the psycho-analyst is of the same kind as that of the child who believes that the sound he hears is coming out of the disc which he sees revolving. The disc is only manifesting its power to react to the stimulus of the needle, and the result is a set of vibrations that the ear interprets as sound. So in consciousness, the psychical centres are manifesting their power to react to certain stimuli, and the concomitant of this reaction is consciousness. Such a view of the nature of mind has no place for the unconscious, or subconscious

mind. There may be in the cortical centres millions of distinct records, but they are records only and not mind. To speak of them as "subconscious mind" is only to introduce confusion into our conceptions of mental operations. It is similar to calling the impressions in a gramophone record "sub-music"; it is of no practical value and quite erroneous.

The psycho-analysts are also in error in holding that a so-called subconscious suppressed complex can act as a pathogenic agent, disturbing the course of normal mentalization. Tanzi and Lugaro (42) have dwelt on this error and fully exposed it. Freud and his followers have in this matter been led astray by giving an illegitimate extension to a well-known phenomenon observed in cases of hysteria. Their symbolic interpretation of dreams is, I maintain, altogether outside the field of science, for they have discussed the subject without reference to the known facts regarding the psychical centres. Dreams are essentially chaotic; during sleep, the wide stream of consciousness has ceased to flow and the surface is still and smooth, but a local ruffling is produced by the dropping in, as it were, of a tiny pebble. There has been an accidental stimulation of an association centre, with its necessary concomitant effect of consciousness. This very partial and localized stimulation is due to toxic or physical actions that affect the centres directly, or indirectly, through distant centres in anatomical and physiological connection with them.

The psycho-analysts can also, I maintain, be shown to have attached a highly exaggerated importance to repression of the sexual instincts in the causation of neurasthenia.

Further, their so-called psycho-analysis has as much right to be regarded as an analysis of the mind as, to give a simple concrete example, the dipping of a litmus paper into ammoniacal liquor is entitled to be called an analysis of this very complex fluid. Credulity and self-deception, to a degree incredible if we had not their own ingenuous records from which to form a judgment, enter into all their operations.

Lastly, psycho-analysts have laid themselves open to just censure by ignoring the fact that many of the morbid pheno-

mena with which they deal are due to toxic actions, which require investigation in each case and which can often be remedied by appropriate measures. If the phenomena they discuss were merely matters of academic interest, their conduct might be regarded as venial, but they are really dealing with a very painful form of human suffering, lamentably common, that involves not only the happiness but often the very lives of their fellow-men. I am well aware, from the evidence of very many cases that have come under my observation, how utterly inadequate their methods of treatment are to relieve the sufferings of the genuine neurasthenic, which are dependent upon neurotoxic actions, for the most part bacterial, and generally amenable to specific methods of treatment.

While insisting upon the importance of bacterial toxæmia as a cause of neurasthenia, I recognize that there are also mental factors of an essential kind. Psychic traumatism plays a very important part in the pathogenesis of many different disorders of the nervous system. It may act directly by modifying the structure and therefore the reactivity of important psychical centres, but it may also act by inducing disorders of metabolism, which in turn entail grave acute or chronic toxæmias. This mental factor requires, however, to be dealt with in a very different way from that in which it is handled by the psycho-analyst.

There is a rather important point that requires to be alluded to and put aside before I deal with the chronic infective conditions that I have found in cases of neurasthenia. Most persons afflicted with this malady suffer from intestinal stasis and its consequent toxæmia. The toxins absorbed from the colon in these cases are no doubt varied in nature and origin, but they are chiefly formed by the action of saprophytic bacteria upon the food residues. Absorbed in excess of the amount that can be destroyed, they produce lassitude, mental depression, slight degrees of mental confusion, more or less severe headache, and sleeplessness or drowsiness. All cases of neurasthenia accompanied by intestinal stasis are aggravated by absorption of these toxins. It is, I believe, an error to regard any case of neurasthenia as

dependent upon intestinal stasis alone. There are always pathogenic factors of much greater moment.

I have records of 110 cases of neurasthenia that I have investigated bacteriologically. In some of the earlier ones anaerobic methods were not applied. It is therefore difficult to frame exact statistics. The study of the last 70 cases has revealed the great prominence of anaerobic diphtheroid bacillus infections of the intestine. At least 63 were found to be suffering from a severe infection of this kind. Hundreds of controls in which no anaerobic diphtheroid bacilli could be found in the stools testified to the abnormality of the presence of these bacilli, at least in very large numbers, in the intestinal tract. The evidence that anaerobic diptheroid bacilli are a cause of neurasthenia lies in the phenomena observable when therapeutic immunization is carried out. It has been demonstrated very frequently that these patients may be intensely sensitive to the vaccine, and that indeed all the neurasthenic symptoms can be aggravated at will by overdoses. Further, it has been found that, in most cases, great benefit results from continued immunization with doses just sufficient to produce a reaction. The cases that do not do well have generally some other factors at work that cannot be influenced by the immunization. Aerobic diphtheroid bacilli occur in the intestine more rarely as neurotoxic infections. Aerobic or anaerobic diphtheroid bacillus infections of the prostate gland, or of the uterus, cause an intense form of neurasthenia. Infections of the bladder by diphtheroid bacilli are commonly associated with well-marked neurasthenia. Chronic infection of the respiratory tract by the influenza bacillus is another cause of neurasthenia in some people. Nine of the 110 cases in my statistics were of this nature, and the relation of the infection to the neurasthenia was sufficiently established by the successful results of therapeutic immunization. Chronic pneumococcus infections of the intestine or respiratory tract may, in some people, cause a severe type of neurasthenia. Eight cases of this kind are included in my statistics. A hitherto entirely obscure cause of neurasthenia, as well as of more grave nervous disorders, is chronic intestinal infection by an anaerobic streptothrix. Less important

causes are chronic infections by *streptococcus pyogenes* and *streptococcus fæcalis hæmolyticus*. It should be added that all cases of chronic gonococcus infection of the urethra or Fallopian tubes present well-marked neurasthenic symptoms which are relieved by suppression of the infection.

Many of the symptoms that the psycho-analyst regards as the expression of the struggles of a suppressed complex are really due to the constant ragging of the nervous system by bacterial toxins that tend to fix themselves in the neurons. Various parts of the nervous system may be affected, the peripheral nerves as well as the centres. Neuralgias, paræsthesias, hyperæsthesias, exaggerated reflexes, constant feelings of fatigue, irritability and mental depression are some of the many effects entailed by the circulation of these toxins throughout the body. Phobias, of the suppressed complex origin of which the psycho-analyst is so sure, are exaggerations of protective instincts which are present in normal measure in others. They are dependent upon increased excitability of the nervous centres, resulting from the same causes as the exaggeration of the deep and superficial reflexes in the same patient.

I advise the thorough investigation by anaerobic and aerobic methods of all possible seats of infection in cases of neurasthenia. The commonest types of chronic infection have been indicated. If any of these are found to be present, therapeutic immunization will, as a rule, bring relief to the patient. In some long-established cases, the pathogenic factors are so complex that therapeutic immunization against existing infections may fail to remove the symptoms. There are many other remedial measures in addition to therapeutic immunization appropriate for cases of this kind, which should be employed in accordance with the principles of modern medicine.

It has been clearly established that many cases of neurasthenia benefit greatly by treatment by a polyvalent vaccine prepared from anaerobic intestinal diphtheroid bacilli. A vaccine of the following composition has been employed :—

1 c.c. = { Anaerobic intestinal diphtheroid bacillus 0·2 mg.
Streptococcus fæcalis hæmolyticus 0·1 mg.

Dose—0·1 c.c. to 2 c.c.

The best evidence that this vaccine is of practical value is the persistence with which, for several years, two or three medical practitioners have asked for fresh supplies.

3. **Exophthalmic Goitre.**—More than two years ago, I recorded observations (46) showing that exophthalmic goitre is a malady superimposed upon neurasthenia due to intestinal infection by diphtheroid bacilli, usually anaerobic. Although this view of the causation of the disease has been fully confirmed by further observations of my own and has been largely utilized in the treatment of the malady by several practitioners, the medical profession in general show no interest in the matter. Learned societies still discuss the pathology and treatment of the disease without a single allusion to its bacterial infective cause, which has been established by evidence as clear as any that could have been furnished by experimental work upon lower animals.

Exophthalmic goitre is evidently determined by the involvement of the cervical sympathetic nervous system in the neurotoxic action of intestinal diphtheroid bacilli, an accident that tends to occur only in a small proportion of those suffering from this kind of infection. It has been shown repeatedly that the patients are intensely sensitive to minute doses of a vaccine prepared from their intestinal diphtheroid bacillus, that all the characteristic symptoms of the disease can be aggravated at will by pushing the dose, and that therapeutic immunization with the same vaccine, given in doses just sufficient to produce a mild reaction, generally results in improvement and ultimate recovery.

I have investigated fifteen cases. These show that a diphtheroid bacillus infection of the intestine is always a prominent feature of the disease. With rare exceptions, the bacillus is anaerobic. The intestinal flora is generally otherwise also abnormal. Colonies of *streptococcus fæcalis hæmolyticus* are usually present in very high proportion. Pneumococcus infections also occur with considerable frequency. The urine is almost always loaded with acrobic and anaerobic diphtheroid bacilli.

I would urge that in cases of this kind a systematic bacteriological investigation of the intestinal and urinary flora should be made, by aerobic and anaerobic methods, on

hæmoglobin media, and that treatment by therapeutic immunization should be carried out in accordance with the results of the investigation.

4. **Disseminated Sclerosis.**—Observations have recently been recorded that tend to show that this disease is caused by a spirochæte. A few cases that I have investigated by bacteriological methods have, however, furnished some evidence that it is another malady superimposed upon neurasthenia due to anaerobic diphtheroid bacillus infection of the intestine, which may extend to the urinary tract. In some persons the neurotoxins would seem to have a selective action upon certain portions of the central nervous system, which, for reasons unknown, are specially vulnerable. Some of the cases I have investigated recovered under therapeutic immunization with autogenous vaccines, and, in one in which the treatment was carried out by myself, the administration of excessive doses more than once intensified all the characteristic symptoms for several hours. It is possible that in this disease something must be allowed for the particular type of infecting diphtheroid bacillus, as there are certainly many distinct pathogenic species.

The case treated by myself was that of a lady teacher who had all the signs of disseminated sclerosis, and was contemplating giving up her work, upon which she depended for her living. I found that both urine and stools were loaded with a purely anaerobic diphtheroid bacillus of unusual morphological characters. Immunization was continued for over a year. As already indicated, focal reactions, manifested by severe nervous disturbances, occurred on several occasions, especially during the first few weeks of treatment. Ultimately, the bacilli could no longer be found in the urine and all signs of disseminated sclerosis had disappeared.

Another case was that of a young lady in whom the features of neurasthenia and disseminated sclerosis were clearly represented. The urine was loaded with anaerobic diphtheroid bacilli very similar to those in the preceding case. Under therapeutic immunization, carried out by her own doctor, she made a slow but complete recovery, and remains well.

I would therefore urge that cases of disseminated sclerosis should be investigated bacteriologically, and that, if similar anaerobic diphtheroid bacillus infections are found in the urinary and intestinal tracts, therapeutic immunization with autogenous vaccines should be carried out.

5. **Tabes Dorsalis.**—The medical profession, guided by its accepted authorities, is quite satisfied that infection by the *spirochæta pallida* is a sufficient explanation of the causation of tabes dorsalis, and it refuses to consider any additional factors that have been regarded by some as taking a part in the pathological process. For many years I have maintained, on the ground of bacteriological and therapeutic observations, that there is an important bacterial factor, consisting usually in an infection of some portion of the genito-urinary tract by neurotoxic diphtheroid bacilli. The toxins of the invading bacteria, passing up the lymphatic channels, in part reach the posterior roots of the spinal chord (already affected in common with the rest of the central nervous system by the syphilitic morbid process) and determine their progressive degeneration. In support of this view there is the evidence of constant severe bacterial infection of the male urethra in cases of tabes dorsalis, the occurrence of toxic reactions in the form of an attack of lightning pains after the administration of an excessive dose of the vaccine, and the complete arrest of the disease by therapeutic immunization in a series of cases. These facts have been recorded, but, as I have indicated, the medical profession, intolerant of any explanation of tabes dorsalis that would appear to weaken the syphilitic hypothesis, refuses to have anything to do with such views and therapeutic recommendations. I am confident that some day it will be recognized that these views are correct, and that an incalculable amount of human suffering might have been avoided but for the stolid prejudice or indifference of those who were bound in honour to do all that knowledge allows for the relief of the sufferings of their patients.

Two observations bearing on this question are worth mention here. It has been shown by an observer, who knew nothing of the opinions of myself and those who were associated with me in some of the investigations to which I

have referred, that some syphilitic gummata will not heal under anti-syphilitic treatment alone, but clear up rapidly when there is added to this treatment therapeutic immunization against diphtheroid bacilli with which they are infected. The case is exactly comparable with that of tabes dorsalis. The second observation concerns the case of a patient whom I saw for a short time several years ago. This man had had syphilis, but he did not suffer from tabes dorsalis, nor had he any chronic infection of the urethra. He showed, however, well-marked ataxia of the upper limbs, affecting chiefly the right side. There was a history of previous phthisis in the right apex, from which he recovered on going to South Africa. When I saw him, he had chronic bronchitis, and I found that his sputum was loaded with pneumococci. Now, this is a neurotoxic bacterium, and it is not, I think, a very strained hypothesis that its toxins, spreading up by the lymphatics to the cervical region, had caused progressive degeneration of the sensory fibres of the cervical nerves rendered vulnerable by the previous action of syphilis.

I have had opportunities of investigating bacteriologically only twenty-six cases of tabes dorsalis. In most of them the disease was very advanced, and the patients suffered from grave secondary infections of the bladder, two circumstances that rendered any very satisfactory result from therapeutic immunization out of the question. Among these twenty-six cases there were, however, six in whom the disease, though quite clearly marked, was yet in a somewhat early phase of its development, and in which there were no serious secondary bacterial infections. All of these six cases had severe diphtheroid bacillus infections of the genital tract, and in every one the progress of the disease has been arrested by corresponding therapeutic immunization.

Mental Disorders

The part played by infections in the causation of mental disorders has already been given general consideration in Chapter II. I have here to deal with the bacterial toxic factors in some of the main forms of insanity, and the

application of therapeutic immunization as a remedial measure.

For many years I have endeavoured to investigate these problems under considerable difficulties. It must be obvious to anyone with medical knowledge, whether he has special experience of mental diseases or not, that investigations of this kind require the sustained observation of individual cases. There must be, first, the careful clinical examination of the patient, then a sufficiently complete bacteriological investigation, and, lastly, the intelligent carrying out of therapeutic immunization and the observation of its effects. When I commenced this investigation many years ago, I pointed out to the Board of the Laboratory of the Scottish Asylums the necessity of my personal observation of cases in a hospital, and made the definite request that they should provide the facilities. With this request they did not see their way to comply, and I have therefore had to carry on as best I could under conditions that have very rarely fulfilled the minimum requirements for such research. During the period of the war, when all asylums were under-staffed, assistance of any value from those in charge of the cases was seldom obtainable. Occasionally it was possible to make a sufficiently complete bacteriological investigation, but only very rarely was it possible to get therapeutic immunization properly carried out. At best, investigations made in this way do not fulfil the requirements of systematic observations carried out from beginning to end by one person. Only cases in a mental hospital, set aside for scientific observation, can completely satisfy the conditions necessary for researches of this kind. The cases that have served me best have been a few of incipient mental disorder that did not require to be sent to an asylum, and which were treated either by myself or by a practitioner experienced in carrying out therapeutic immunization.

1. **Acute Insanities.**—Acute forms of insanity, ranging from the milder types of amentia to acute delirium, are essentially of toxic origin. From the clinical side, very striking evidence supporting this view has been obtained by Lewis C. Bruce (17). The toxæmia is not in all cases

bacterial, although in the great majority of cases it certainly is. That acute forms of insanity may occur in the course of such definitely infective diseases as influenza, puerperal fever, erysipelas, typhoid fever, pneumonia and smallpox has long been recognized. It must be admitted, however, that much uncertainty remains regarding the exact part played by bacterial infection in such cases. These infections do not necessarily cause insanity: in fact, they comparatively rarely do so. We must therefore recognize that the pathogenesis of acute insanity has other factors than infection. Much must be allowed to individual predisposition to fix toxins in the cortical nerve cells, to the state of the patient's defences against toxins and against bacterial invasion, to the severity of the toxæmia, and to the degree of neurotoxicity of the bacterial products, which varies with different strains of the same bacterium. In many cases there are mixed infections, and a particular combination of toxins may, in some instances, determine a strong neurotoxic action.

The recorded observations would warrant the conclusion that the bacteria that most commonly cause acute insanity are *streptococcus pyogenes*, pneumococci, *bacillus typhosus* and *bacillus influenzæ*. Of still greater practical importance than all of these put together are, however, in my opinion, several species of neurotoxic diphtheroid bacilli. Aerobic forms seem to be of most frequent occurrence, and the most potent, although anaerobic types also play a large part. In some cases of this kind there is what may be termed diphtheroid saturation. Nearly all the mucous membranes are infected by the bacilli. These are being passed into the blood-stream and poured through the kidneys into the urine. Some cases of this kind of infection have served to demonstrate a fact of great practical importance—namely, that psychical traumatism may increase the vulnerability of the nerve-cells and so determine the onset of the acute phase of the mental disorder.

I have made a bacteriological investigation in thirty-four cases of acute insanity. In twenty of these, aerobic or anaerobic diphtheroid bacilli seemed to be important infective agents determining the mental disorder. Severe infection by pneumococci occurred in five of the cases.

Streptococcus pyogenes infection appeared to be responsible for the illness in three, the bacillus of influenza also in three, *bacillus proteus* in two and anaerobic gonococci also in two. In most of the cases there were mixed infections. In all, the bacterial attack was extremely severe and widespread, and there were generally sufficient grounds for regarding it as the cause of the patient's mental illness. The following are some examples :—

A lady, eighteen years of age, in the course of a few weeks developed maniacal symptoms with disorientation and delusions. Bacteriological investigation showed that the urine and genital tract were loaded with aerobic diphtheroid bacilli. Vaccines were prepared and administered, and it was found that each dose was followed by a passing increase in the mental disturbances. With careful regulation of the dose, the patient steadily improved for two months. Then the Great War broke out. The patient had recently spent a long period at school in Germany, and had made many friends there. She was consequently greatly distressed. Her mental trouble quickly became aggravated, and it was thought best to send her to an asylum. Therapeutic immunization was continued, and she made a complete recovery. It may be said that she would have recovered without immunization. All that is insisted upon, however, is the fact of the severe infection by a bacillus of known neurotoxic powers and the definite reaction that followed the administration of minute doses of a corresponding vaccine. Three years later, the patient had a similar attack. The same infections were found, but, in addition, by anaerobic methods, it was ascertained that there was an extremely severe diphtheroid bacillus infection of the intestine. She was sent to an asylum, where therapeutic immunization was carried out, and again she seemed to respond to the vaccines, and was discharged in perfect health after a few months. The patient's doctor, satisfied that the neurotoxic diphtheroid chronic infection was the cause of the attacks, and desiring to use prophylactic measures, has continued to give her a dose of her vaccine at intervals of about a month. Under this treatment the patient has now for over four years remained in excellent health. I think this case illustrates the fact of the increased vulnerability of the cortical nerve cells to toxins when their functional activity is disturbed by very strong emotions. There were circumstances in the patient's history that made it almost certain that an emotional cause precipitated a second attack, as well as the first. Three years after recovery from the second attack, when she was being immunized regularly against the bacillus that was believed to be an essential cause of her illness, an important crisis did occur in her life, but she passed through it without another attack of insanity.

A man, aged twenty-six, was admitted to one of the district asylums suffering from acute mental disorder. It was found that his stools and urine were loaded with anaerobic diphtheroid bacilli. The doctor in charge of the case treated him with autogenous vaccines, and reported that the patient made a fairly rapid and complete recovery.

A young lady had for some weeks shown mental disturbances that took the form of excitement, foolish conduct, incoherence and delusions. She was sent to a nursing home. A bacteriological investigation showed that the intestinal flora consisted chiefly of anaerobic diphtheroid bacilli, and that the lower genital tract was loaded with aerobic diphtheroid bacilli with unusual morphological characters. The chief interest of the case lies in the fact that a first dose of only 0·02 mg. of a vaccine prepared from the aerobic bacillus was followed within two days by an acute maniacal outburst, on account of which she had to be sent to an asylum. I believe that this was a case in which the patient was extremely hypersensitive to the toxin of the diphtheroid bacillus. The dose of vaccine administered resulted in the liberation of a large amount of toxin in the infective focus, in consequence of which the neurotoxic action was greatly increased.

The cases of puerperal insanity included in the series showed one or other of three types of uterine infection—namely, by *streptococcus pyogenes*, pneumococci, and aerobic diphtheroid bacilli. The following is an example of acute insanity apparently due to intestinal infection by pneumococci :—

A man, aged thirty-one, had been confined to an asylum for several years on account of recurrent attacks of mental confusion, each of which had lasted for many weeks. During these attacks he suffered from diarrhœa. I found that he had a severe intestinal infection by pneumococci. He was treated by therapeutic immunization, and the doctor in charge of the case subsequently reported that the patient had quickly improved, both in his mental and in his physical condition.

Most of the cases investigated showed evidence of mixed infections, always of severe degree.

A female patient in one of the District Asylums suffered from acute confusional insanity. Cultures from the intestinal tract, nasopharynx, nasal passages and urine yielded profuse growths of aerobic and anaerobic diphtheroid bacilli. There was also evidence of nasopharyngeal infection by an anaerobic strain of *streptococcus fæcalis hæmolyticus* (always an important pathogenic bacterium

when occurring in this situation), and the only coliform bacillus in the intestine was the bacillus of Friedländer.

Another female patient in a District Asylum was admitted suffering from an attack of acute insanity. She was found to have cystitis due to an infection of the bladder by *streptococcus fæcalis* ; there were severe infections of the nasopharyngeal region by *bacillus proteus*, *staphylococcus pyogenes albus* and *streptococcus fæcalis hæmolyticus*. There was an intestinal infection by *bacillus proteus*.

A young woman suffered from symptoms which her medical attendant regarded as those of incipient insanity. I found that there were severe intestinal infections by anaerobic diphtheroid bacilli, *streptococcus pyogenes*, and pneumococci, and a menstrual blood swab revealed the presence, not only of abundant anaerobic diphtheroid bacilli but also of anaerobic gonococci.

Every one of the thirty-four cases of acute insanity that I have investigated suffered beyond all question from bacterial infections of great severity. This I attest with the comparative experience of similar investigations carried out by the same methods in many hundreds of cases of other forms of illness. The few cases that I have been able to follow out have yielded evidence that the infections present were essential factors in the causation of the mental disorder. This evidence consisted in the occurrence of distinct reactions to the vaccines, improvement under treatment and the comparative paucity, or complete disappearance, of the pathogenic bacteria on repetition of the bacteriological examination, when the patient had recovered partially or completely. I believe that a great deal could be done to promote recovery in cases of this kind by therapeutic immunization based on accurate bacteriological investigation. Great care must be taken that the toxæmia is not unduly increased by the administration of doses of vaccine sufficient to liberate a large amount of toxin from the infective focus. This warning is especially important in cases of diphtheroid bacillus infection. Probably the initial dose of this kind of vaccine should never, in this form of illness, exceed 0·001 mg. It is doubtful if vaccines can help at all in cases of the nature of those that I have termed "diphtheroid saturation." How the toxæmia is to be diminished in such cases is a problem for the future. Much greater hope lies, I think, in the use of an anti-serum than in the administration of

an autogenous vaccine, when all the tissues seem to be bathed in the toxic products of disintegration of these bacilli.

2. **Manic-Depressive Insanity.**—It is generally recognized that the group of mental disorders that have, as their chief and essential feature, the exaggeration of the affective tone that characterizes the large manic-depressive group, or the affective psychoses, do not have a common pathogenesis. The type of case in which mania and melancholia alternate is probably best explained as dependent upon a disturbance of the bodily chemistry, to which the unfortunate patient is rendered liable by constitutional defect. Another type of case is represented by the simple mania of some patients suffering from exophthalmic goitre, certainly a disease due to infection, and by the chronic melancholia of some cases of bacillus coli cystitis.

A simple instance of a purely toxic mental depression is occasionally to be observed in persons who, after extraction of a number of teeth, have the misfortune to suffer from invasion of the lacerated tissues by a neurotoxic type of *streptococcus pyogenes*. The absorption of the toxins of neurotoxic strains of this bacterium may, in such circumstances, cause very distressing mental depression, which passes off when the local infection subsides.

On the analogy of these cases, we should be prepared to find that many other infections may also, in some persons, give rise to grave disturbances of the affective tone. Bacteriological investigation in a series of cases, especially of mental depression, in many instances not so severe as to constitute insanity, has sufficiently confirmed this view. The study of these cases has yielded evidence that there is very commonly a bacterial toxic basis of the mental disorder, and that accurate therapeutic immunization may be an effective means of treatment.

The infecting bacteria do not essentially differ from those found in cases of acute insanity. Aerobic and anaerobic diphtheroid bacilli, *streptococcus pyogenes*, *bacillus coli communis*, *streptococcus fæcalis hæmolyticus*, *bacillus influenzæ* and anaerobic streptothrices are perhaps the pathogenic species that may most frequently be found as the agents of

a severe infection in such cases. The following are some typical examples :—

An asylum patient suffered from chronic melancholia and hallucinations of hearing. I found that she had an infection of the gums and nasopharynx by the bacillus of influenza, complicated by the common streptococcus and *micrococcus catarrhalis* infections of post-nasal catarrh and pyorrhœa alveolaris. She was treated by therapeutic immunization against each infection and made a complete recovery.

A young man in one of the District Asylums was subject to frequently occurring attacks of mania. His gums were much inflamed and yielded profuse growths of the bacillus of influenza and *streptococcus pyogenes*. There was also a well-marked intestinal infection by anaerobic diphtheroid bacilli. He was treated by therapeutic immunization against these pathogenic bacteria and made "a splendid recovery."

A man of middle age suffered from severe neurasthenia, mental depression and inability to concentrate his thoughts, on account of which he was obliged to give up his work as a school teacher. I found that he had very severe nasal infections by an anaerobic *streptococcus fæcalis hæmolyticus* and diphtheroid bacilli. His urine was loaded with diphtheroid bacilli, but the intestinal flora appeared to be normal. Under therapeutic immunization against these infections, he steadily improved, and made a complete recovery in the course of two or three months.

A woman, thirty-eight years of age, had suffered for fourteen months from mental depression, insomnia and visceral delusions. Bacteriological investigation showed that the urine was loaded with anaerobic diphtheroid bacilli, which were present also, although in comparatively small numbers, in the intestinal tract. The nasopharynx was the seat of a severe infection by *streptococcus anginosus*. The most remarkable feature of this case was the occurrence of a cystitis associated with anaerobic diphtheroid bacilli. The patient was treated by therapeutic immunization at the asylum of which she was an inmate, but I received no report of her subsequent progress.

Many other cases of melancholia could be cited in which aerobic or anaerobic diphtheroid bacillus infections of the intestinal and urinary tracts seemed to be the cause of the patient's nervous illness. At least half of the cases of this class that I have investigated have had infections of this nature. I have investigated a sufficient number of cases to show that very many in this group suffer from severe chronic bacterial infections incompatible with health. The treatment of the asylum cases not having been in my own

hands, it has only very occasionally been possible for me to follow them out as the investigation required.

3. **Dementia Præcox.**—As this form of insanity is very common and is rarely recovered from, its many victims become a charge upon the community for the rest of their lives and crowd every asylum. So rare are recoveries that they are generally recorded (43). If the disease could be prevented or cured, these institutions would be more than half emptied in a few years' time. Hence the unsolved problems of its pathology, prevention and successful treatment are matters of great importance. This has been very fully recognized in the United States, where a most laudable systematic effort is being made by Dr Bayard Holmes to stimulate research upon the subject, one of the measures adopted being the publication of a journal, *Dementia Præcox Studies*, devoted specially to this one form of mental disease.

There is still a remarkable lack of definite knowledge regarding the causes and pathogenesis of dementia præcox. It is well to bear in mind that the malady is clinically a process of mental disorganisation, associated with a series of more or less severe attacks of insanity. Most of the theories regarding the nature of the disease fail to account for these repeated spurts displayed by the pathological process. The theory that the disease is due to disorder of the endocrine glands remains vague and unsupported by any conclusive evidence. The view that it is a sort of neuronic decay dependent upon a germinal lack of durability, which is observable also in the constituents of the testes and ovaries, has little satisfactory evidence to support it, much of that which is adduced being capable of other interpretations, whilst it is completely out of harmony with the fact that the disease develops as a series of more or less acute attacks.

The errors of the theory of psychic trauma advocated by the psycho-analysts have been very thoroughly exposed by Tanzi and Lugaro (42. Vol. ii., pp. 510-511). Psychic dissociation is a fundamental fact of the malady, and it is therefore natural that "dissociated complexes" of every kind should be readily discoverable in the patients. The psycho-analyst finds in the effects of this disease the cause of the disease itself.

An infective theory is held by many, but, like the other views, it lacks definiteness and supporting evidence. In my judgment, it is, however, the only theory that in the least accords with the known facts. It would account especially for the frequent spurts exhibited by the malady. Wherever there is a contest between chronic infective elements and the defensive forces of the body, the former from time to time get the upper hand, and acute disturbances are always the result. A flare-up of a chronic infection, with its attendant increase of toxins in the blood, might quite well explain an exacerbation of the mental disturbances in this disease. That dementia præcox is essentially a manifestation of tuberculosis is, I believe, quite an error. Tuberculosis is certainly common in the victims of dementia præcox, but it is not constant, and its prevalence can be explained as a result of the general lowered resistance of the patients to bacterial infection.

For several years I have endeavoured to investigate the bacterial infective conditions in cases of this kind, and, although the observations have been made under great difficulties, it has been possible, I think, to throw some light on the matter. It is first to be noted that inherited predisposition to dementia præcox is a definitely established fact. I think this inherited predisposition manifests itself chiefly and essentially as a special vulnerability of the most highly evolved cortical neurons to various toxins, and especially to those of bacterial origin. If we can admit this special vulnerability in certain stocks, and particularly in certain individuals belonging to these stocks, the rest is not difficult to explain, for it can be shown that every victim of dementia præcox in its progressive phase is suffering from extremely severe bacterial infections, such as are never found in sane persons without grave impairment of their physical health. This fact is constant in my experience.

The question next arises if these infections are a mere result of the patient's mental disorder, or its cause. Apart from data derived from animal experiments, which in the nature of things are hardly procurable, the proof lies with the accurate bacteriological analysis of the infections from which a case is suffering, and the subsequent observation of the

occurrencc of focal reactions to suitable doses of autogenous vaccines, and of the results of systematic therapeutic immunization in a series of cases. The second part of this investigation cannot be properly carried out except upon patients in a well-equipped hospital. Until recently, I have had no opportunities of making such observations, save in a few privatc cases. This portion of the investigation is therefore still incomplete. As already indicated, the bacteriological investigation of a series of cases has, however, established, I believe, the fact that these patients, during the periods of their acute illness, constantly suffer from severe bacterial infection, and it has also justified some conclusions regarding the forms of infection that occur.

I have been unable to find any evidence pointing to the occurrence of a special infection. There would appear to be several types of neurotoxic bacterial infection to which the cortical nerve cells of these patients show extreme sensitiveness. Three forms of infection are specially prominent—namely, by neurotoxic diphtheroid bacilli, by pneumococci, and by the essentially neurotoxic anaerobic streptothrices of the intestine.

Cases of the first type generally have severe diphtheroiduria, but on further investigation it is always demonstrable that a similar infection occurs elsewhere, most commonly in the intestinal tract, nasal passages and genital tract. Many of these cases show the condition that I have described as diphtheroid saturation. This is not confined to dementia præcox, but no one suffering from it escapes nervous disorders. There is at least severe neurasthenia. It may determine an attack of acute insanity from which the patient recovers. Individual predisposition and power of resistance are so varied that it is not by any means impossible that the samc bacterial toxæmia should in one person induce only a disorder of the functional action of the neurons from which he can recover, whilst in another it should entail a very similar disorder which leaves the physical basis of the mind permanently damaged. Something must, however, be allowed to differences in the neurotoxic characters of the invading bacilli.

The pneumococcus type is fairly common. The seat of the

infection is the intestinal tract. The patients are generally very anæmic.

The streptothrix type has been recognized only somewhat recently, but it is already evident that it is of very frequent occurrence. It is associated with glycosuria, which, however, may be only slight and occasional, though often it is very well marked. There is an intestinal infection of the same kind as that already described as being constantly associated with diabetes mellitus.

These types may be combined. Thus, an anaerobic streptothrix infection of the intestine is frequently associated with an anaerobic diphtheroid bacillus infection, and the latter with chronic pneumococcus infection. Other important chronic infections in various situations are always present. The most common are those by *streptococcus pyogenes*, *staphylococcus pyogenes*, aerobic diphtheroid bacilli, the bacillus of Friedländer and the bacillus of influenza. There are remarkable differences in the kind and in the localization of these associated infections. Thus, some cases have severe pyorrhœa alveolaris, generally dependent chiefly upon infection by *streptococcus pyogenes*, whilst in other cases the gums and teeth are in excellent condition. Chronic infection of the fauces and nasopharynx are also very common, and, in these, *streptococcus pyogenes* is likewise generally prominent. Nasal infections, especially by aerobic diphtheroid bacilli, are very common, but cases occur in which the nasal passages are free from infection.

Evidence has been obtained that, if the chronic bacterial infections in cases of dementia præcox in the early stage are properly treated by therapeutic immunization, the patient benefits both in bodily and in mental health. It is futile to expect any restoration of mental capacity in advanced cases, in which the association centres have been gravely damaged. When the importance of bacterial infection as a cause of dementia præcox becomes fully realized, the cases will be investigated and treated at an early stage. The exact measure of control that we may thus be able to exert over the malady has yet to be determined. The following are some illustrative cases :—

1. A man, twenty-three years of age, was admitted to Roxburgh District Asylum with the typical features of dementia præcox in its early phase. The urine was loaded with diphtheroid bacilli. Ulceration of the gums around the few remaining stumps was found to be associated with infection by the bacillus of influenza and *micrococcus catarrhalis*. There was a severe infection of the nasopharynx by *streptococcus pyogenes*. The nasal passages were loaded with aerobic diphtheroid bacilli. Colonies of *streptococcus fæcalis hæmolyticus* predominated in the intestine. This case was investigated some years ago, when anaerobic methods were not systematically employed, but experience shows that diphtheroiduria is always associated with anaerobic diphtheroid bacillus infection of the intestine. I prepared autogenous vaccines from all the pathogenic bacteria found. A course of immunization was given by Dr Carlyle Johnstone and Dr Charles A. Crichlow, who reported that the patient steadily improved and was discharged cured after six months. He had been resident in the Asylum for three months before treatment was begun without any improvement taking place.

2. A female patient in a District Asylum, suffering from dementia præcox, was found to have severe infection of the intestine by pneumococci and anaerobic diphtheroid bacilli, and of the gums and fauces by *streptococcus pyogenes*. This was a typical example of the combined diphtheroid bacillus and pneumococcus type.

3. A woman, aged forty, was admitted to a District Asylum with an illness of recent onset which was diagnosed as dementia præcox. She was thin and very anæmic. The gums were intensely inflamed along the edges of the teeth. A bacteriological investigation showed that they were the seat of severe infections by *streptococcus pyogenes* and *micrococcus catarrhalis*. In cultures from the stools, there were about one hundred colonies of streptococci to one of coliform bacilli. The streptococcus colonies were found to consist of *streptococcus pyogenes*, pneumococci and *streptococcus fæcalis hæmolyticus* in about equal proportions. The coliform bacillus was the bacillus of Friedländer. Pneumococci were also found in the urine, which, further, contained abundant anaerobic diphtheroid bacilli. The nasal passages were loaded with the bacillus of Friedländer. This is a typical example of the pneumococcus type, complicated by severe oral and intestinal infections by *streptococcus pyogenes*.

4. A young woman, recently admitted to the Royal Edinburgh Asylum and regarded as a typical case of dementia præcox, presented very similar infective conditions of great severity. The gums were in excellent condition, but the nasopharynx was much inflamed and a swab yielded profuse growths of *streptococcus pyogenes* and *micrococcus catarrhalis*. The intestinal flora consisted of pneumococci, *streptococcus pyogenes*, the bacillus of Friedländer and anaerobic diphtheroid bacilli.

5. A young woman, aged twenty, recently admitted to one of the District Asylums, showed the typical features of dementia præcox.

The gums, which were swollen and congested, and bled readily, yielded profuse growths of *streptococcus pyogenes* and *micrococcus catarrhalis*. The stools in aerobic culture gave a normal flora except that there were some colonies of aerobic diphtheroids; in anaerobic culture abundant growths of a streptothrix were obtained. The urine contained a considerable amount of sugar; it yielded rather profuse growths of aerobic and anaerobic diphtheroid bacilli.

6. A young man, recently admitted to an Asylum on account of symptoms diagnosed as those of dementia præcox, showed a remarkable condition of chronic infection of the fauces and pharynx. Cultures yielded profuse growths of *streptococcus pyogenes*. The intestinal flora, in aerobic culture, was almost normal; there was no excess of streptococci. In anaerobic culture, abundant streptothrix and anaerobic diphtheroid bacillus colonies were obtained. The urine gave a slight, but distinct, positive reaction to Fehling's test.

5. Diseases of the Skin and Subcutaneous Tissues

Diseases of the skin present a large field for the application of therapeutic immunization. Nevertheless, it must be admitted that, in many cases, lesions, almost certainly of bacterial origin, prove refractory to this form of treatment. Various reasons may be assigned for this limited amount of success. Incomplete bacteriological diagnosis accounts for some of the failures. For example, special strains of staphylococci, difficult to distinguish from others that are also present, but of vital importance in the case, may not be included in the vaccine. In other instances, failure is probably due to the fact that leucocytes and anti-bacterial bodies cannot obtain ready access to the infected tissues on account of sclerosis resulting from long-standing inflammatory action. A similar partial protection from leucocytes and antibodies is enjoyed by *bacillus acnes* and its attendant staphylococci, owing to the fact that they are lying within sebaceous glands.

1. **Boils and Carbuncles.** — These acute inflammatory lesions are dependent upon the pathogenic action of *staphylococcus pyogenes* in the skin and subcutaneous tissues. *Aureus* is by far the most frequent, *albus* and *citreus* being found only occasionally. In eighteen cases, I found *albus* only once. One of the cases, however, yielded, instead of *staphylococcus pyogenes aureus*, only a mannite non-

fermenting white staphylococcus and an anaerobic diphtheroid bacillus. In some very severe cases of acne of the back the large pustules that form are virtually indolent boils; I have examined many of them and have never been able to obtain from them any other staphylococcus than a mannite non-fermenting one.

The infection in cases of boils is essentially from the surface of the skin. In cases of recurrent boils the superficial layers of the skin in certain regions must be regarded as infected by staphylococci which are ready to invade when aided by various slight causes, such as pressure and trivial injury.

Therapeutic immunization can be of much service by circumscribing boils, and hastening their ripening; in some instances it will serve to abort them. In cases of recurrent boils, it is generally very successful in arresting the trouble. The initial dose is 0·05 mg. and the amount should, in the course of about ten doses, be gradually carried to 0·5 mg. at least, and, if possible, to 1 mg. Some cases are very sensitive to the toxin at first, and in these it is necessary to persevere with a small dose until the hypersensitiveness is overcome. In all cases of recurrent boils, an effort should be made to disinfect the special areas of the skin in which the staphylococcus invasion takes place. Corrosive sublimate, dissolved in absolute alcohol to the strength of 1 in 1000, washed over the parts, with the aid of a swab of cotton-wool, and allowed to dry, has, in my experience, proved very effective.

2. **Abscesses.**—Local suppuration in the various regions in which it may occur is dependent upon the action of one or more of a large variety of pathogenic bacteria. These include *staphylococcus pyogenes*, *streptococcus pyogenes*, pneumococci, *streptococcus anginosus*, *streptococcus fæcalis*, *bacillus coli communis*, *bacillus typhosus*, *bacillus pyocyaneus*, the gonococcus, *bacillus tuberculosis*, *actinomyces* and various anaerobic bacteria that occur as wound infections. Chronic abscesses are generally tubercular.

In most cases, therapeutic immunization can certainly be of great service, if based upon accurate bacteriological investigation. It cannot take the place of surgical measures, but it is often capable, to a most valuable extent, of helping

the surgeon to obtain a good result. The general principles that must guide one in carrying out therapeutic immunization in such cases have already been laid down. The valuable aid that this method of treatment can give in these cases is well illustrated in a series of observations recorded by Henry A. Craig (25).

3. **Adenitis.**—In acute forms of adenitis the kind of help that may be afforded by therapeutic immunization is precisely that which has just been defined in regard to cases of abscess. The chronic forms are generally tubercular, and a word may be said about the employment of vaccines in their treatment. The general opinion seems now to be that the best results are obtained by the use of bovine and human tuberculin together, in the doses already indicated in Chapter X., section 9. Allen (3) has very rightly insisted upon the importance of being on the look-out in these cases for secondary invaders, usually staphylococci or streptococci, and of making cultures from the material obtained by puncture from glands that are threatening to suppurate. By timely immunization against such secondary infections, suppuration may often be prevented.

4. **Ulcers.**—Very many forms of ulceration are in large measure dependent upon chronic bacterial infection of the tissues, and in such instances therapeutic immunization, based upon accurate bacteriological analysis of the flora, has an extremely useful application. It does not interfere with the employment of any other measures that may seem necessary, and experience has shown that it can often change the whole aspect of the lesion and produce rapid healing. The infecting bacteria that may be present includes staphylococci, and streptococci of various types, *bacillus tuberculosis*, *bacillus coli communis*, *bacillus pyocyaneus*, diphtheroid bacilli and sporing anaerobes. With the last, which fortunately do not often occur in civil practice, it seems impracticable to deal by immunization, because of the difficulty there is in obtaining a sterile vaccine.

The value of giving doses of polyvalent vaccine of *bacillus coli communis* in cases of varicose ulcer seems to be well established.

5. **Erysipelas and Cellulitis.**—Erysipelas is always due to

invasion of the tissues by a special type of *streptococcus pyogenes*. Cellulitis is likewise frequently dependent upon local infection by *streptococcus pyogenes*, but probably of a different type. It may also be due to invasion of the tissues by other bacteria, including *staphylococcus pyogenes*, the pneumococcus, *bacillus pyocyaneus* and *bacillus coli communis*. Henry A. Craig (25) has recorded successful results from therapeutic immunization in an extensive series of cases, both of erysipelas and cellulitis. Allen (3) refers to Ross and Johnson's extensive observations in the vaccine treatment of cases of erysipelas in America. They found the most striking effects lay in the rapid subsidence of toxæmic symptoms, such as mental unrest, delirium and profound malaise. The effects upon the local condition and upon the temperature were also highly satisfactory. Their initial dose in severe cases was 10,000,000, in less severe cases, 20,000,000. The dose was generally repeated next day, and thereafter every second day. Allen recommends a preliminary dose of 50 c.c of anti-serum, followed by a stock or autogenous vaccine "as soon as the toxic symptoms are under control." He gives 10,000,000 of an ordinary vaccine, or 25,000,000–50,000,000 of a sensitized vaccine, if it is available.

6. **Wound Infections.**—The bacteria that commonly occasion sepsis in wounds are especially *streptococcus pyogenes*, *staphylococcus pyogenes* and *bacillus pyocyaneus*, the pathogenic action of which can generally be checked by accurate therapeutic immunization. Post-operative infections are specially suitable for this form of treatment, as the conditions are generally ideal for investigation and further observation. Henry A. Craig (25) records the results of treatment in sixty cases and states that all were materially benefited.

In war, anaerobic bacteria derived from the soil are frequently added, giving rise to gangrene. According to the authoritative conclusions of the Medical Research Committee (Special Report Series, No. 39, by W. Bullock and others. See *The Lancet*, 3rd Jan. 1920), the important anaerobic bacteria that occur in these cases and the frequency of their incidence are as follows:—*B. Welchii*, 70%; *Vibrion septique*, 16%; *B. œdematicus*, *B. fallix* and *B. histolyticus*, 14%. The

modern specific treatment of infections by these micro-organisms is not by therapeutic immunization, but by means of "antigas gangrene serum," the results obtained with which in France are said to have exceeded all expectations.

7. **Acne.**—This common malady is primarily due to infection of the sebaceous glands by *bacillus acnes*. There are cases, certainly somewhat rare, in which this is the sole infection. As a rule, staphylococci occur within the glands along with the bacillus. It has been too readily assumed that these staphylococci are of the species *pyogenes*. I have regularly tested many strains with mannite and have found that in the large majority of cases the reaction is negative. *Staphylococcus pyogenes aureus* and *albus* do, however, occur with some frequency and generally in cases with very acute pustulation. On the other hand, there are cases in which all efforts to find *bacillus acnes* fails; these are almost always cases in which the sebaceous glands are infected by a mannite non-fermenting staphylococcus. Other forms of secondary infection occasionally occur, especially by *streptococcus pyogenes* and by diphtheroid bacilli that can be distinguished from *bacillus acnes*.

The secretion of the infected gland is perverted and thickened, and in consequence the openings become blocked. Behind the obstruction the gland gradually distends, until the septic contents some day rupture into the surrounding tissues. Acute local inflammation and the formation of a pustule follows. Efforts to express a comedo are very apt to lead to similar infection of the surrounding tissues and acute local inflammation. Massage may have the same result, and it is, I think, rarely wise to resort to it until the patient has been immunized, when it can be used with great benefit. Most patients who suffer from acne have chronic intestinal or respiratory tract infections which aggravate the skin trouble. In order to obtain a satisfactory result it is generally necessary to investigate and treat these associated maladies. Constipation, whether combined with intestinal infection, or not, always aggravates acne.

Most cases of acne yield satisfactorily to properly directed therapeutic immunization; a small proportion prove refractory. It is best to prepare an autogenous *bacillus acnes*

vaccine, but a stock vaccine often acts quite well. It is essential to prepare autogenous vaccines from the associated infecting bacteria. A course of ten or twelve weekly inoculations is generally sufficient to effect a cure which, however, may not be complete. The dose of *bacillus acnes* may begin with 0·05 mg., and should be carried up to 1 mg.; the initial dose of a mannite non-fermenting staphylococcus is the same, but the final dose may often be carried up to 2 mg. As there are frequently several varieties of staphylococci at work, it is a good plan to make a second examination after five or six weeks and to prepare a new vaccine from the strains that remain. The first three or four doses given will generally result in rapid ripening of indolent pustules, and it is well to warn the patient that this will occur. Much valuable practical advice regarding the treatment of acne will be found in a paper by Allen in the *Journal of Vaccine Therapy*, Jan. 1912, as well as in this authority's books (1 and 3).

8. **Seborrhœa.**—The bacteriology of this form of skin disease is essentially similar to that of acne. Some authorities believe, however, that the bacillus is not identical with the one that produces the latter malady. Therapeutic immunization should be carried out on the same lines as those just recommended for acne.

9. **Psoriasis.**—This is one of several skin diseases that has baffled all efforts of the bacteriologist to discover an infective cause. Recently, some observations have been recorded that would seem to show that the malady is due to a spirochæte. I have made very many cultures from patches of psoriasis, in the acute initial stage, on various media of different degrees of acidity. Those made under the best conditions have remained sterile; only when cultures were made from chronic lesions did various common bacteria develop. These, I believe, have no essential connection with the disease. In several cases in which I have investigated the intestinal flora, I found a very unusual and extreme prominence of diphtheroid bacilli, generally aerobic.

10. **Eczema.**—Although this skin disease is of local infective origin, the bacterial attack seems always to be

rendered possible only by some co-existing disturbance of general nutrition. This is frequently due to chronic infection of the intestinal tract. For example, in the case of a boy who had suffered from eczema since infancy, I found that the lesion of the skin was associated with severe infections by *staphylococcus pyogenes aureus* and *streptococcus pyogenes*, and that the urine and stools were also loaded with the latter bacterium.

The infecting bacteria are almost constantly those represented in the case just cited—namely, *staphylococcus pyogenes albus*, *aureus* or *citreus* and *streptococcus pyogenes*. The association of the two seems to be the rule, but the staphylococcus may occur alone. Therapeutic immunization will rarely be of any avail unless other existing maladies are successfully diagnosed and treated. Possible infections of the alimentary and respiratory tracts should be carefully investigated.

Many of the patients are extremely sensitive to autogenous vaccines prepared from the bacteria infecting the skin, and, in these, it is imperative to continue with minute doses until this hypersensitiveness is overcome. In the case of the boy just mentioned, this condition was extreme, and it was at first thought that therapeutic immunization was going to fail. After reduction of the dose to about one-tenth, however, he immediately began to improve and ultimately made a good recovery.

A lady, over seventy years of age, had suffered for more than a year from eczema of the hands, arms and face. She had a succession of severe relapses in which the right auricle and the external auditory meatus were prominently involved. The first cultures yielded only profuse growths of *staphylococcus pyogenes albus*, but, during a relapse marked by an erythematous swelling of the ear, abundant growths of *streptococcus pyogenes* were also obtained. The patient was intensely sensitive to autogenous vaccines, no more than 0·025 mg. of staphylococci and 0·0025 mg. of *streptococcus pyogenes* being tolerated, without a state of collapse occurring on the following day. This hypersensitiveness was overcome in the course of about six weeks, and progressively higher doses were then well borne. The patient sustained a distinct chill during a spell of cold weather and the eczema of the hands relapsed, but the inflammation completely subsided within a week. In the course of about three months the patient made a complete recovery, and gained in weight and vigour.

11. **Impetigo.**—Allen (1) states, on the authority of M'Watters, that the infective cause of this malady is *staphylococcus pyogenes aureus* in the cases that begin as a scab formation, and the *streptococcus* (*pyogenes*) in those that begin with a bulla. According to this observer, the malady yields rapidly to the respective vaccines.

12. **Sycosis.**—This is due to invasion of the hair follicles by *staphylococcus pyogenes*. In some cases, *streptococcus pyogenes* is also found. I have investigated only three cases, each of which was due to infection by *staphylococcus pyogenes*. All did well under treatment by autogenous vaccines. One of the cases is of special interest. It was that of a gentleman who had been on the Continent on business, and, before leaving the port of embarkation to return home, he went to a barber's shop and had a shave. I saw him a few days afterwards. A large area of the chin and cheeks was intensely inflamed, and every hair seemed to be standing in the centre of a well of pus. I started treatment immediately with a stock *staphylococcus pyogenes* vaccine and gave him a solution of corrosive sublimate in alcohol (1 in 1000) to apply from time to time to the affected part. Cultures yielded pure growths of *staphylococcus pyogenes aureus*. Immunization was continued with autogenous vaccines, but was soon stopped, for, under the local and specific treatment, the trouble had entirely disappeared in little over a week.

Chronic cases will generally require a fairly long course of immunization.

13. **Lupus.**—This is to be regarded as dependent upon a local tubercular infection, associated with secondary invasion, especially by staphylococci and streptococci. Modern methods of local treatment are generally successful, and therefore therapeutic immunization is not usually called for. It may, however, be carried out on the principles already laid down for immunization against other chronic tubercular infections. The secondary infections should always be investigated.

Robert Robertson (26), has treated several cases successfully with tuberculin, combined with *bacillus coli communis* vaccine. The action of the latter can only be regarded as

pharmacological in such cases, but nevertheless it seems to be extremely valuable.

14. **Chilblains.**—The treatment of this malady by vaccines is one of the curiosities of therapeutic immunization. Robert Robertson has found that immunization against *staphylococcus pyogenes aureus* in people who are subject to chilblains is an effective remedy and a sure preventive. As with the use of a *bacillus coli communis* vaccine in chronic ulcers, and as an aid to tuberculin treatment, we have clearly to deal with an action that is not specific (for there is no infection to combat), but pharmacological.

15. **Conjunctivitis.**—This is a convenient place in which to consider the application of therapeutic immunization to acute and chronic forms of conjunctivitis and closely related maladies. It may here be mentioned that chapters of great practical value, based upon wide experience, dealing with vaccine therapy in relation to Diseases of the Eye, are contained in Allen's early and most recent books (1 and 3)

Acute Conjunctivitis may be due to infection by the Koch-Weeks bacillus, the gonococcus, the pneumococcus, *streptococcus pyogenes*, *staphylococcus pyogenes*, the bacillus of Friedländer, *bacillus coli communis*, *bacillus pyocyaneus* and some other species. Therapeutic immunization against such infections by means of autogenous vaccines has given very good results in the hands of numerous observers.

Chronic Conjunctivitis may be caused by the diplobacillus of Morax-Axenfeld, staphylococci, the bacillus of Friedländer, and *bacillus tuberculosis*. Personally, I am somewhat sceptical about the Morax-Axenfeld bacillus being anything more than a very common type of diphtheroid. Several cases that I have investigated have yielded growths of a bacillus of this kind with metachromatic granules. In one of the patients two distinct types were present. The relation of these diphtheroids to the chronic inflammation has been established by the observation of typical reactions and by the successful results of therapeutic immunization. Chronic infections of the conjunctiva by the bacillus of Friedländer and the tubercle bacillus are said to be very resistant to specific treatment.

6. Diseases of Bones and Joints

1. **Acute Periostitis and Osteomyelitis.**—These diseases are dependent upon local infection by *streptococcus pyogenes*, *staphylococcus pyogenes*, pneumococci, *bacillus typhosus*, etc. Although their treatment is at present almost exclusively surgical, the time must come when therapeutic immunization will be recognized as a necessary aid in order to secure the best results.

2. **Chronic Periostitis and Osteomyelitis.**—Some cases are due to tubercular infection, but secondary infections, especially by *staphylococcus pyogenes* and *streptococcus pyogenes*, are common. *Bacillus coli communis*, *bacillus pyocyaneus*, diphtheroid bacilli and pneumococci have also been found. Here also therapeutic immunization has its place as an agent supplementary to surgical measures. A combination of human and bovine tuberculin seems to give the most satisfactory results. Other infections, whether primary or secondary, can be properly treated only by autogenous vaccines, after careful bacteriological investigation by aerobic and anaerobic methods.

3. **Acute Arthritis.**—This may be dependent upon local infection by such pathogenic bacteria as *staphylococcus pyogenes*, *streptococcus pyogenes*, *streptococcus fæcalis* (*hæmolyticus*?), pneumococci, *bacillus coli communis*, *bacillus pyocyaneus*, *bacillus typhosus* and the gonococcus. Treatment is, in the first place, medical and surgical, but therapeutic immunization unquestionably has its valuable application as an aid. In this category is to be included acute gonococcal rheumatism. The chronic form is one of the types of rheumatoid arthritis. Acute rheumatism due to joint infection by the gonococcus has frequently been successfully treated by vaccines, on lines similar to those already described for acute urethritis. Hale White and Eyre (18) were among the first to apply this measure with success to cases of gonococcal rheumatism. Fraser and Duncan (*The Lancet*, 31st Jan. 1920) have reported four cases treated successfully by vaccines injected intravenously.

4. **Acute Rheumatism, Rheumatic Endocarditis and Chorea.**—

The various forms of rheumatism are maladies that affect many organs and tissues, but, as some of their chief manifestations occur in the bones and joints, they are considered in this section for convenience.

Poynton and Paine's *micrococcus rheumaticus* is regarded by some as the bacterial cause of acute rheumatism and closely related maladies, whilst others are unable to accept this view.

My own observations favour the conclusion that the chief bacterial cause of simple rheumatism, in its acute, subacute and chronic forms, is a streptococcus of the *pyogenes* group, frequently showing a preference for anaerobic conditions of growth. It is generally, but not always, hæmolytic. No ascertained biochemical reactions are of service for its differentiation from other types of *streptococcus pyogenes* with other pathogenic actions. In chronic cases, the type of *streptococcus pyogenes* is certainly not always the same, differing especially in size and in the firmness of the cohesion of its chains, but I have not sufficient data to judge if this is also the case in acute infections.

It seems to me at least probable that in many instances the streptococcus described as *micrococcus rheumaticus* has really been a long-chained streptococcus that has been mistaken for a short-chained one, for its long-chained character is not always easy to demonstrate, unless broths containing fresh serum are used, and, even then, only if special care is taken in the manipulation of the deposit in preparing a film for examination.

Very successful results in the treatment of cases of acute rheumatism and chorea have been recorded by Buchanan (22) from immunization with a stock vaccine of *micrococcus rheumaticus*. On the other hand, W. S. Harrison has reported negative results in eight cases, and Emery (32), in commenting on his paper, expresses the opinion that the evidence in support of this bacterium being the cause of rheumatic fever "is certainly incomplete and inconclusive, and tends, indeed, strongly in the opposite direction."

This is a class of case that my official position has given me few opportunities of studying, and therefore I refrain from making any recommendations regarding therapeutic

immunization beyond the one that arises from the view just expressed, which is based chiefly upon the study of subacute and chronic cases.

5. **Simple Chronic Rheumatism.**—The division of chronic rheumatic affections into simple rheumatism and rheumatoid arthritis is made here partly for convenience of description, but also because there is a fundamental difference in the infective causes. Clinically, the latter malady is distinguished especially by irregular overgrowth of the opposed cartilages of the joints, in consequence of which free movement at the articulations becomes impossible. Simple chronic rheumatism may be due to the pathogenic action of several different bacterial species, and all of them may occur in rheumatoid arthritis, but in the latter disease there are always added special infections that appear to determine the proliferation and degeneration of the cartilage cells.

The clinical manifestations of simple chronic rheumatism are, as is well recognized, very numerous, including pain, swelling and stiffness of the joints, muscular rheumatism, neuritis, neuralgias, lumbago, sciatica, iritis, etc. For the past ten years, I have almost continuously made bacteriological investigations in cases of simple chronic rheumatism and of rheumatoid arthritis, and have carried out therapeutic immunization in a large proportion of them. Although many points remain undetermined and obscure, I believe that the essential causes of both forms are now known. Accurate therapeutic immunization based upon this knowledge is generally successful, but has its necessary limitations, and also its occasional apparently impassable barriers to encounter.

At the present day, simple chronic rheumatism is generally attributed to the action of *micrococcus rheumaticus*. This theory, upon which the composition of many stock vaccines on the market is founded, is, I believe, quite inadequate. As I have already stated, the streptococcus, to which this name is given, and which is beyond all question an important cause of rheumatism, is, in my opinion, almost certainly really a long streptococcus belonging to the *pyogenes* subgroup But this is by no means the only bacterium capable

of causing chronic rheumatism. The bacteriological and therapeutic investigations that I have made into the question lead me to conclude that simple chronic rheumatism may be due to one or other of at least six different species, and that generally two or more of these occur in every case. These species are as follows :—

(1) *Streptococcus pyogenes.*—Only some types cause rheumatism, and these cannot as yet be distinguished absolutely from other types by any known criteria. Both anaerobic and aerobic rheumatic strains are common, and the former are of special importance and must always be looked for.

(2) *Streptococcus anginosus.*—Only certain strains cause rheumatism.

(3) *Streptococcus salivarius,* Inulin Fermenter.—This is only an occasional, but nevertheless important, cause of simple rheumatism.

(4) The bacillus of influenza, occurring as a chronic infection. This is a definitely proved cause of chronic rheumatism.

(5) Pneumococci, probably of different type from those that cause rheumatoid arthritis.

(6) An anaerobic diphtheroid bacillus, distinguished especially by the fact (as far as the evidence at present goes) that it grows freely only on a very alkaline medium (+6). It has the form of a straight homogenous rod with terminal metachromatic granules. It occurs chiefly in the intestine.

The most common seats of infection are the gums, the alveoli, the tonsils, nasopharynx, bronchi and intestine. The association of rheumatism with pyorrhœa alveolaris is well recognized, and yet it is true that only a small proportion of persons suffering from this disease of the gums ever has a twinge of rheumatism. Whether the latter malady develops or not depends on the nature of the infections, their severity and the rheumatic tendencies of the patient. There may be severe pyorrhœa without any bacterial infections of a kind that can cause rheumatism. Deep infection of the alveoli is generally associated with pyorrhœa, but

it is of the utmost practical importance to know that it may occur without pyorrhœa and around the roots of teeth that, on simple inspection, appear perfectly sound. I have been able to prove this in several cases in which deep infection was suspected, although the teeth looked sound, and in which teeth removed with precautions against contamination were immediately submitted to bacteriological investigation. In several cases of this kind I have obtained profuse growths of an anaerobic *streptococcus pyogenes* from the deeper portion of a root. Chronic infection of the tonsils can generally be recognized on simple inspection, but it is important to bear in mind that severe infections of the nasopharynx may, though rarely, occur without the patient suffering any inconvenience. It is therefore never sufficient to accept the patient's opinion that there is no post-nasal catarrh as a reason for not investigating this region. *Streptococcus pyogenes* infection of the intestine may cause chronic rheumatism, but only exceptionally. Still more rare is the occurrence of a rheumatic type of *streptococcus anginosus* in the intestine. Chronic infections by the bacillus of influenza occur in the gums, tonsils, nasopharynx and lower respiratory tract.

It is possible that the anaerobic types of *micrococcus catarrhalis*, which may be found very commonly in association with *streptococcus pyogenes* in rheumatic cases, are sometimes a contributing cause of simple rheumatism, but this micrococcus occurs so frequently as a catarrhal organism apart altogether from rheumatism that there is some difficulty in estimating its possible importance in this disease.

In all cases of chronic rheumatism a very extensive bacteriological investigation is necessary. Every possible seat of infection must be explored, and intestinal aerobic and anaerobic cultures should never be omitted. Many of these patients suffer from anaerobic diphtheroid bacillus infection of the intestine, and are consequently more or less distinctly neurasthenic. Moreover, as already indicated, there are good grounds for believing that there is a particular type of anaerobic diphtheroid bacillus that is not only neurotoxic in its action, but also a cause of chronic rheumatism. In cases with a strong neurasthenic element due

to causes of this nature, the amount of pain suffered by the patient is often out of all proportion to the severity of the actual rheumatism. Some of these cases respond to therapeutic immunization against their intestinal diphtheroid bacilli, but others remain uninfluenced by it and become the despair of the physician. Rheumatoid arthritis still more frequently presents examples of this complication by neurasthenic pain. These neurasthenic complications, which, however, are not always resistant to specific treatment, are the chief impassable barriers that I have referred to as being placed in the way of successful therapeutic immunization in some cases of chronic rheumatism.

The average case generally requires three or four separate vaccines. These may be combined in one dose in suitable amounts, according to the known limits of dosage of each element. Immunization generally requires to be prolonged. A course of twelve weekly doses has commonly to be supplemented by five or six additional high doses given at intervals of a fortnight or three weeks. Hypersensitiveness may be encountered at the beginning of the course, and it is then of the utmost importance to reduce the dose and to provoke only mild reactions. As a rule, however, there is no hypersensitiveness, and the dose may be pushed up rapidly, without the patient suffering more than a slight degree of malaise next day. If the bacteriological diagnosis has been correct, and the vaccines are properly prepared and administered, there is generally steady improvement. The following are some illustrative cases :—

A doctor suffered from severe chronic rheumatism, affecting especially one shoulder and extending down the arm. His gums at the side of one or two of the teeth were swollen and red, and bled easily. Cultures yielded profuse growths of an anaerobic *streptococcus pyogenes*. Under therapeutic immunization against this bacterium, the patient's rheumatic trouble quickly subsided.

A surgeon had suffered for several months from very severe rheumatism, affecting the neck, shoulders and arms, and rendering him unfit for his work. His teeth and gums were in excellent condition, but there were well-marked symptoms of chronic post-nasal catarrh. Investigation showed that the nasopharynx was severely infected by several types of *streptococcus pyogenes*, as well as by *streptococcus anginosus* and an anaerobic *micrococcus catarrhalis*.

There was also an intestinal infection by anaerobic diphtheroid bacilli. Under therapeutic immunization against these pathogenic bacteria he made a slow, but satisfactory, recovery.

A medical student had suffered for three years from severe rheumatism beginning in the arms and extending to numerous joints, including the knees. He had had acute rheumatism when a child. The chronic malady had become so severe that he was faced with the prospect of having to abandon his studies. His teeth and gums were in excellent condition, and he had no signs of post-nasal catarrh. There was no intestinal disorder. In searching for an infective focus, I observed that he had several curious livid patches about his fauces. Cultures from these yielded profuse growths of *streptococcus pyogenes*, chiefly anaerobic strains, and of an anaerobic *micrococcus catarrhalis*. Therapeutic immunization was carried out against these infections. In the course of twelve injections, the dose of a polyvalent *streptococcus pyogenes* vaccine was carried from 0·02 mg. to 0·4 mg., and that of the anaerobic *micrococcus catarrhalis* vaccine from 0·05 mg. to 0·4 mg. The patient soon began to improve, and by the end of the course was quite free from rheumatism, only a little stiffness of some of the joints remaining.

A young lady had recently suffered from two separate attacks of subacute rheumatism, with rise of temperature, whilst spending a long holiday in the country. On returning to town, she was threatened by a third attack, and therefore sought advice. The shoulders and neck were chiefly affected. The joints of the fingers and wrists were distinctly swollen. She had previously been treated by therapeutic immunization for chronic post-nasal catarrh. This had been cured, and there was now no return of it. The teeth and gums were in good condition. Both tonsils were, however, much inflamed. Aerobic cultures showed abundant growths of *staphylococcus pyogenes aureus*, but no streptococci. Anaerobic cultures yielded, from one side only, profuse growths of *streptococcus pyogenes*, and, from the other side, *micrococcus catarrhalis*. Immunization was carried out against these three pathogenic bacteria, and the patient steadily improved, and seemed to be making a complete recovery. Towards the end of the course, however, she suddenly developed an attack of acute tonsillitis. This was found to be associated with severe infection by an aerobic *streptococcus pyogenes* and *streptococcus anginosus*, two species that had not been present at the time of the examination made three months before. This attack of tonsillitis was followed, after a week, by some recurrence of rheumatic pain, though of a different type from that previously experienced. New vaccines were prepared, and it was found that she was unduly sensitive to them, the ordinary initial doses being followed in two days by a toxic reaction in the form of rheumatic pain. Under continued immunization the malady disappeared and the tonsils became normal in appearance.

An elderly gentleman had suffered for some weeks from severe

iritis, for which he had had only local treatment. He was sent to me with the request that I should endeavour to find a bacterial infective cause for the mischief. All of his teeth had been extracted some years before, with the exception of one canine, which had been left for the support of a denture. The surrounding gum was swollen and inflamed. I made cultures from this infective focus and obtained profuse growths of pneumococci and *streptococcus pyogenes*. The tooth was extracted, and, under therapeutic immunization against these bacteria, the iritis rapidly cleared up and the patient remained well.

An elderly lady had suffered for several years from severe rheumatism affecting especially the neck and shoulders and one knee. Results of cultures made from the gums and nasopharynx seemed insufficient to account for the trouble. Nearly all of the teeth were present, but several were crowned. I insisted that the patient was suffering from alveolar infection of some of them. One was extracted by a dentist, and handed to me for investigation. I found that there was severe infection of the root, chiefly by anaerobic bacteria. These consisted of *streptococcus pyogenes*, *streptococcus fæcalis hæmolyticus* and *micrococcus catarrhalis*. In aerobic cultures, colonies of *streptococcus anginosus* were abundant. Therapeutic immunization was carried out against these bacteria, and the patient made a complete recovery from her rheumatic trouble, and was enabled to retain her remaining teeth.

A married lady had for four months suffered from a very painful type of rheumatism affecting, according to her own account, every part of the body. Nevertheless, she was able to walk quite well, and the joints showed only slight and rather doubtful indications of swelling. There was severe gingivitis along the line of the upper incisors, and cultures yielded profuse growths of aerobic and anaerobic types of *streptococcus pyogenes*, *streptococcus anginosus* and anaerobic *micrococcus catarrhalis*. Other possible sites of infection were investigated with unimportant results, excepting that the intestinal tract was found to be loaded with anaerobic diphtheroid bacilli of two types, one of ordinary characters flourishing on a + 18 medium, and the other growing well only on a + 6 medium. Therapeutic immunization was carried out, first, against the infections of the gums, without any benefit. Then a vaccine prepared from the common type of anaerobic diphtheroid bacillus was added. Nevertheless, the patient always came back saying she was worse. She continued, however, to be able to go about, and walked without lameness. The second type of anaerobic diphtheroid bacillus was discovered on a further examination being made, but it was impossible to test the effect of a vaccine, as the patient was sent to one of the resorts for rheumatic subjects by her doctor. I regard this case as one of those in which the neurotoxic action of anaerobic diphtheroid bacilli is an intensifier of rheumatic pain. Neurasthenia is the main fact in such cases, and, whilst therapeutic immunization helps in

many of them, other measures are generally nccessary, in order to restore the patient to health. Nevertheless, a more recent case, with very similar infective conditions, has shown that the addition of an anaerobic diphtheroid bacillus vaccine, prepared from an intestinal bacillus that grows well only on a +6 mcdium, and has the special morphological characters described, may be followed by rapid cessation of pain and complete recovery.

6. **Rheumatoid Arthritis.**—The investigations that I have carried out during the past ten years seem to me to justify the conclusion that the infective elements that determine the development of rheumatoid arthritis are at least two in number. They are a special type of pneumococcus and anaerobic gonococci. Roughly, the former accounts for about 60% of the cases, and the latter for about 40%. The two infections may be, and, indeed, often are, combined. They may be associated with any of the bacteria that cause simple chronic rheumatism. Thus, there is a wide range of difference in the bacteriological picture from case to case. Each patient must be made the subject of systematic examination, every possible seat of infection being investigated and a careful search being made for every known primary or contributing bacterial cause of the disease. Such investigations are extremely laborious, but, if therapeutic immunization is to be applied successfully to a case, they are absolutely necessary. Some of the results that can be obtained are well worth all the trouble they entail. Unfortunately, there is a residuum of cases, larger than in simple chronic rheumatism, in which a neurasthenic element, due almost always to neurotoxic diphtheroid bacillus infections of the alimentary or genito-urinary tract, seems to impose an impassable barrier to success. In advanced cases, the distortion of the joints is of course permanent; we can only hope to arrest the pathological process and so to relieve, to some extent, the patient's sufferings. The day will come when all of these cases are treated early by accurate therapeutic immunization, and the bed-ridden, immobilized and tortured sufferer will be as rare as a case of fully developed myxœdema. It may be added that unless there is an early complete change in the present attitude of the medical teacher and of the general practitioner towards therapeutic

immunization, this very desirable day will probably not dawn during the present century. Rheumatoid arthritis is an active infective disease, to be combated only by scientific measures directed against the bacterial attack, and all the present authoritative teaching on the subject about disorders of metabolism and deficiencies of endocrine glands is simply erroneous speculation.

It is very desirable that the type of pneumococcus associated with rheumatoid arthritis should be specially investigated and accurately defined. It has been impossible for me to give time to this matter. The conclusion that certain cases of rheumatoid arthritis are due to the action of a bacterium of this kind is based upon the intense sensitiveness of the patients to the toxin, the observation of focal and toxic reactions, and the success of therapeutic immunization in many cases. I have not produced, or tried to produce, rheumatoid arthritis in lower animals by means of this pneumococcus, but I have frequently, to my regret, intensified for many days the characteristic sufferings of several patients, through injecting a dose of not more than 0·001 mg. of the dead bacteria.

Investigations are also required regarding the characters of the anaerobic strains of gonococci that can be isolated from a large percentage of cases of rheumatoid arthritis. Such infection occurs very commonly without the development of rheumatoid arthritis, and the question arises whether a special type is the cause of the disease, or whether the patient's reactive qualities are the determining factor. That this bacterium is an essential cause of the malady in the cases in which it occurs has been clearly proved by the action of autogenous vaccines. Experience has shown that this type of case is very favourable as regards the prospects of an arrest of the progress of the disease, which, however, commonly produces much irreparable distortion of the affected joints. Early recognition of the infective cause and prompt treatment by therapeutic immunization will, in course of time, be the orthodox practice in these cases.

The following are some notes of actual observations. The first four cases are examples of the pneumococcus type. The

next four are of the gonococcus type, and the last four are cases of combined infection :

A married lady, who had a long history of previous illness on account of which hysterectomy had been performed, began to suffer from severe rheumatoid arthritis, affecting especially the feet. I found that there was an extensive infective focus in the gum in the region of the lower incisors. Cultures from the swollen and inflamed tissues yielded profuse growths of *streptococcus pyogenes*, pneumococci, and aerobic diphtheroid bacilli. The only coliform bacillus in the intestine was the bacillus of Friedländer, and anaerobic diphtheroids were very abundant. Under therapeutic immunization against these pathogenic bacteria, the rheumatic trouble was completely arrested, and the patient has since remained well.

A single lady of middle age had suffered for several years from severe rheumatoid arthritis, affecting chiefly the hands, which were greatly swollen, very painful, and much distorted. She had chronic nasal and post-nasal catarrh. All of the teeth had been removed, and the gums were healthy. From a nasal swab, confluent growths of *staphylococcus pyogenes aureus* were obtained, and a post-nasal swab and sputum yielded very abundant growths of *streptococcus pyogenes, streptococcus anginosus, streptococcus fæcalis hæmolyticus, micrococcus catarrhalis*, and pneumococci. Other regions were investigated with negative results. Under a course of therapeutic immunization against these pathogenic bacteria the patient improved. A second examination was made, and it was found that the only pathogenic bacteria remaining were *staphylococcus pyogenes aureus* and *streptococcus pyogenes*, the latter being probably of a different type from that previously isolated. Immunization against these infections was continued, and the patient further improved, ultimately making a very satisfactory recovery, although, of course, the deformity of the hands remained.

A lady had suffered for several months from severe rheumatism, affecting especially the arms, and showing the distinctive characters of early rheumatoid arthritis. Most of the teeth had been lost, but the gums appeared in good condition, except for slight pyorrhœa around one incisor. Cultures made from the nasopharynx yielded profuse growths of pneumococci and of an anaerobic *streptococcus fæcalis hæmolyticus*. Therapeutic immunization against these was carried out, but the patient did not improve. I urged that expert advice should be obtained regarding possible deep infection of the remaining teeth. In the course of his examination, the dentist discovered a sinus leading from an alveolus into the antrum. He sent me a swab taken from this sinus ; it yielded profuse growths of pneumococci and also some colonies of *streptococcus anginosus*. After local treatment by the dentist and some further therapeutic immunization, the patient greatly improved, and the subsequent

history of the case has shown that the grave disablement with which the patient was at one time threatened has been averted.

A single lady, of middle age, had suffered from rheumatic pain for nine years, dating from an attack of acute influenza. Several joints of the arms and legs had become greatly swollen ; she was able to get about only with much difficulty, and could do little with her hands. There was chronic post-nasal catarrh ; the teeth had all been extracted and the gums were firm, but they showed some livid patches. The flora of the nasopharynx included the bacillus of influenza, *streptococcus pyogenes* and the bacillary form of *micrococcus catarrhalis*, all of which were abundant. Cultures from a livid patch in the mouth yielded profuse growths of a pneumococcus and of *streptococcus pyogenes*. The nasal passages were loaded with *staphylococcus pyogenes albus* and diphtheroid bacilli. Under therapeutic immunization against these infecting bacteria, the patient made an excellent recovery. She exhibited in a very striking fashion the hypersensitiveness of these cases to a pneumococcus vaccine, with characteristic toxic pain reaction occurring from two to three days after the injection. A dose of only 0·001 mg. produced a violent reaction of this kind lasting nearly a week. Tolerance of the pneumococcus toxin was, however, soon obtained, and a fifteenth and last dose of 0·04 mg. caused no reaction. The progress of the malady was, in this case, completely arrested, and pain ceased to be felt, excepting in the feet after prolonged standing or walking. I believe that, in this case, the chronic infection by the bacillus of influenza had an important action in stimulating the other infections.

A woman, aged fifty-five, had suffered for over twenty years from occasional attacks of diarrhoea, sometimes lasting several weeks, For three or four years, she had been troubled with rheumatism, affecting especially the hands. There was typical rheumatoid arthritic swelling of many joints. Bacteriological investigation showed that there was a well-marked intestinal infection by an anaerobic *streptococcus pyogenes* and anaerobic diphtheroid bacilli. There was no evidence of cystitis, but in anaerobic culture the centrifuge deposit from the urine yielded abundant growths of diphtheroid bacilli and (on a 0·6 medium) of gonococci. A streptococcus similar to that in the intestine was found in the nasopharynx ; there were no pneumococci. It was concluded that the gonococci found in the urine had been derived from the genital tract. Therapeutic immunization against these pathogenic bacteria was carried out, and the patient's doctor, after some months, reported that her general health was much better, and that the rheumatism had greatly improved.

A single lady, aged thirty-five, had suffered five years previously from a " nervous breakdown." From that time there had been a constant vaginal discharge. She began to suffer from rheumatoid arthritis. When I saw her, there was great swelling and distortion of the joints of the knees, ankles, elbows, wrists and fingers. The teeth and gums

were in good condition, and there was no history of post-nasal catarrh. Bacteriological investigation showed that there was no important abnormality of the flora of the stools, but that the urine was loaded with *streptococcus pyogenes* and diphtheroid bacilli, both of which grew best anaerobically. A menstrual blood swab yielded profuse growths of aerobic and anaerobic diphtheroid bacilli, and, on a + 6 medium, colonies of an anaerobic Gram-negative diplococcus. Like the preceding case, this seemed to be one of gonococcus rheumatoid arthritis, complicated by *streptococcus pyogenes* and anaerobic diphtheroid bacillus infections. The patient improved under therapeutic immunization, but only slowly, the strong neurasthenic element that was present being an unfavourable feature in the case.

A man of middle age had suffered for over a year from severe rheumatoid arthritis, affecting various joints, but chiefly those of the wrist and hand, which were greatly swollen and distorted. There was a history of an attack of urethritis occurring many years previously. It was found that there was a urethral infection by an anaerobic gonococcus. The fauces and nasopharynx yielded profuse growths of *streptococcus pyogenes, streptococcus anginosus, micrococcus catarrhalis* and *bacillus pertussis*. There were no pneumococci. Under therapeutic immunization, great improvement occurred. After treatment for about four months, it was ascertained that the gonococcus had disappeared from the urethra.

A young lady had suffered for two or three years from severe rheumatoid arthritis, affecting nearly all of the joints and causing almost complete crippling, so that she became bed-ridden. There were severe infections of the nasopharyngeal region by *streptococcus pyogenes* and *staphylococcus pyogenes aureus*. The stools and urine were loaded with anaerobic diphtheroid bacilli. No gonococci could be detected at first, either in the urine, in a menstrual blood swab, or in a swab from the uterus. I was not, however, satisfied with these negative results, and as the patient did not improve under immunization against the pathogenic bacteria found, I induced the doctor in charge of the case to send another uterine swab. This yielded abundant growths of an anaerobic gonococcus. Therapeutic immunization was carried out against this infection with only partial success. It was thought advisable to extract some of the teeth. With precautions against contamination, one of these was sent to me for bacteriological examination, and cultures from the root yielded abundant growths of anaerobic *streptococcus pyogenes* and anaerobic *micrococcus catarrhalis*. This case is still under treatment, but it is of interest as illustrating the complexity of the infective factors in some of these patients, and the difficulties they present to complete investigation.

A man of middle age first came under observation six years ago, suffering from severe rheumatoid arthritis, affecting chiefly the hands and ankles. He had chronic post-nasal catarrh and cultures yielded abundant growths of *streptococcus pyogenes, streptococcus anginosus*

and pneumococci. Mannite non-fermenting staphylococci were very numerous in the nasal passages. Under rather protracted therapeutic immunization against these infecting bacteria the patient improved, and was himself quite satisfied with the result. At that time the importance of anaerobic gonococcus infection was not known. Recently, the patient requested further treatment on account of a return of the malady. I found that there was no evidence of the pneumococcus infection having returned, but long-chained streptococci were very abundant in the nasopharynx. A specimen of the first urine passed in the morning was requested, and a culture from this yielded very numerous colonies of an anaerobic gonococcus. It is clear that this had been a gonococcus case from the first, although complicated by pneumococcus and other infections. A second course of immunization resulted in almost complete cessation of rheumatic pain, and freedom from subacute attacks.

A lady, twenty-eight years of age, had suffered for nine years from an extremely severe form of rheumatoid arthritis. All the joints were affected, and the patient was bed-ridden. On the recommendation of a doctor at a well-known watering place, and against the advice of her own medical attendant, all the teeth were removed, but without benefit. The malady had steadily gone from bad to worse. I found that there was a nasal infection by *staphylococcus pyogenes aureus*, and severe post-nasal infections by *streptococcus pyogenes* and a pneumococcus. The only intestinal abnormality consisted in the presence of great numbers of anaerobic diphtheroid bacilli. There was no cystitis. Several menstrual blood swabs were examined. At first, these yielded only confluent growths of a mannite non-fermenting staphylococcus. Only after immunization against all of these infecting bacteria had been continued for a time, and when this mannite non-fermenting staphylococcus had been suppressed, was the fact revealed that the patient had a severe infection by an anaerobic gonococcus. Distinct focal and toxic reactions were obtained to autogenous vaccines of pneumococci and of the gonococcus. It is difficult to say if the patient was much benefited by the long course of immunization. I satisfied myself, by examination of successive swabs, that the gonococcus infection had been suppressed, and then stopped treatment. I believe that the rheumatic processes were actually arrested, but the neurotoxic intestinal infections did not respond to immunization, and neurasthenic pain, interpreted by the patient as rheumatic, consequently continued.

An elderly married lady, who had had no children, suffered from severe rheumatoid arthritis and neurasthenia. She had chronic nasal and post-nasal catarrh, and the flora included a pneumococcus, to a vaccine of which she was intensely sensitive. The interest of the case lies in the fact that the centrifuge deposit from the urine, in anaerobic culture, yielded profuse growths of a gonococcus. The patient had distinct pelvic reactions to a corresponding vaccine, and the diplococcus disappeared under immunization. She unfortunately

also had a severe anaerobic diphtheroid bacillus infection of the intestine, which aggravated her neurasthenic condition. She was, however, unquestionably much benefited by therapeutic immunization.

A young married woman, with no children, had suffered for several years from rheumatoid arthritis, affecting all the joints and rendering her bed-ridden. All the teeth had been extracted. She had severe nasal and post-nasal infections, pneumococci being prominent. To a vaccine prepared from these she was intensely sensitive. A culture made from a uterine swab by aerobic methods yielded only diphtheroid bacilli. Later, the examination of a menstrual blood swab by anaerobic methods revealed the presence of gonococci. She had well-marked pelvic reactions to a corresponding vaccine, and the diplococci ultimately disappeared. The chief interest of this case is that the patient, who was not neurasthenic, became free from rheumatic pain, after a long course of immunization.

It will now be understood that therapeutic immunization in any case of rheumatoid arthritis, if it is to be satisfactory, must be based upon a very exhaustive bacteriological investigation. Every possible source of infection must be explored, and all pathogenic bacteria occurring as the agents of a chronic infection must be dealt with. As a matter of routine, the possibility of there being a gonococcus infection must be investigated with the utmost care.

Therapeutic immunization against a pneumococcus should be begun in these cases with a dose not exceeding 0·0005 mg., and the dose should not be increased until it is made clear that one of this amount does not induce severe pain in the joints in from two to three days. Some patients are so sensitive to this vaccine that it is necessary to give even a smaller dose than that named. It will generally be found that tolerance of the toxin gradually increases. I have frequently been able to carry the dose up to 0·05 mg. When this amount has been reached, the immunization may be stopped. If a tolerance of from 0·5 mg. to 1 mg. of anaerobic gonococcus toxin has been obtained, it will almost constantly be found that the diplococcus has disappeared. When it has been ascertained by cultural examination that this end has been achieved, there is no further reason for continuing the immunization.

In cases complicated by anaerobic diphtheroid bacillus infection of the intestine, it is always worth while persevering

for many weeks with corresponding immunization. Great care should be taken that the presence of the special type to which a rheumatic as well as a neurotoxic action can be attributed is not missed.

7. **Tubercular Disease of Bones and Joints.**—Therapeutic immunization seems to be attended with the largest measure of success in cases of this kind when a combination of human and bovine tuberculin is employed. Allen (1) recommends an initial dose of 0·00001 mg., to be increased gradually to 0·00005 mg., at intervals of seven to ten days. If there are secondary infections, they should be treated by means of autogenous vaccines.

7. Diseases of the Blood and General Infections

1. **Simple Anæmias.**—Most chronic infections entail some degree of anæmia as a minor consequence. The application of therapeutic immunization in such cases would generally be called for on account of other symptoms of a more serious nature. There appears to be no form of chronic infection that causes simple anæmia as its chief pathogenic action.

2. **Pernicious Anæmia.**—As has already been stated in the preceding chapter, I maintain that it is established that this disease is commonly caused by a chronic intestinal infection by a special type of pneumococcus. The toxæmia of such an infection is not, however, necessarily the only possible cause of the disease, the essential feature of which is exhaustion of the proliferative capacity of the bone marrow, which might quite well be brought about by other toxic actions.

Some further account of the thirteen cases I have investigated is required here. All of the patients were in an advanced stage of the disease. In each, the intestinal flora included an intensely hæmolytic short-chained streptococcus, giving the reactions of a pneumococcus. Raffinose was fermented as a rule, inulin less frequently. Many strains showed little or no power to ferment lactose. The salicin test, which was applied only in the more recent cases, was found to be negative. It was possible to try the effect of treatment with an autogenous vaccine in only three of the cases. Each of these showed extreme hypersensitiveness, manifested by an attack

of diarrhœa and great prostration. I was able to make a prolonged study of the effects of vaccines in one, that of a lady who presented the interesting feature of pneumococcus infection, not only of the intestine, but also of the mouth, the tongue being especially affected. A dose of only about 0·006 mg. could be tolerated at first. This was gradually raised to 0·018 mg., in the course of about nine months. During this time the patient improved very much. Subsequently, however, she had several relapses, always distinctly traceable to a chill, and one of these precipitated the fatal result of the illness.

I have observed thirty-four other cases of chronic intestinal infection by pneumococci in persons who did not show the distinctive features of pernicious anæmia. In only eleven of these, however, was the pneumococcus of the intensely hæmolytic type found in cases of declared pernicious anæmia. All of these eleven patients were very anæmic, and two of them suffered from paraplegia, which had developed very gradually. As is well known, the toxic conditions present in pernicious anæmia frequently lead to degenerative changes in the spinal chord, and I think that these two cases of paraplegia may be interpreted as dependent upon the fact that the pneumococcus toxin affected the lateral tracts of the chord earlier than usual. There is no class of infective disease more amenable to therapeutic immunization than ordinary pneumococcus chronic infection of the intestinal tract, and therefore we might expect that infection by the intensely hæmolytic type should be capable of similar successful treatment. The few cases of the kind that I have had an opportunity of treating have confirmed this expectation. Several of these candidates for pernicious anæmia have responded splendidly to the action of autogenous vaccines. In one of the cases, by repeated examination of the intestinal flora I was able to observe the gradual disappearance of the pneumococcus. In my experience, these patients respond to therapeutic immunization quite as well as those who suffer from chronic intestinal infection by the more common types of pneumococci. I therefore believe that the conclusion is justified that if these pneumococcus infections were detected early and treated by specific methods

the occurrence of pernicious anæmia would become almost unknown. If the infection is allowed to continue until it has produced exhaustion of the proliferative capacity of the bone marrow, the patient becomes incapable of effective response to vaccines. These considerations give great practical importance to systematic bacteriological investigation of the intestinal flora in cases of anæmia, especially when associated with attacks of diarrhœa. At the early stages of this form of pneumococcus infection, the patients almost never show any special sensitiveness, and therapeutic immunization proceeds rapidly to a successful result.

3. **Septicæmia.**—Therapeutic immunization has now been applied successfully to general infections of the blood-stream. Allen (1, p. 263; 3, p. 175) deals with the subject at considerable length and records some excellent results obtained by himself. Wynn (31) has also put on record some very successful cases.

The infecting bacteria are various and include *streptococcus pyogenes*, *staphylococcus pyogenes*, pneumococci, *streptococcus fæcalis*, *bacillus coli communis* and *bacillus pyocyaneus*. There is always some infective focus from which the bacteria have invaded, as, for example, the uterus in a case of puerperal septicæmia. Only in a limited number of cases is it possible for the surgeon to deal successfully with the local morbid condition. In some instances attempts to eradicate an infective focus may actually cause septicæmia. For example, I have seen a *staphylococcus pyogenes* septicæmia follow excision of a prostate gland infected by this bacterium.

Reasons have already been given, in Chapter IV., for the belief that it is possible by therapeutic immunization to benefit a patient who is suffering from acute bacterial infection, and the principle advocated is applicable to septicæmia. Allen affirms that antibodies are not formed in the blood-stream, but in the extra-vascular tissues, and this consideration, if in accord with fact, strengthens the case for the application of therapeutic immunization in septicæmia. The rule that must be followed is to give small doses of the corresponding vaccine frequently—that is to say, every twenty-four or forty-eight hours. It may be necessary to

begin with a stock vaccine, but an autogenous one should always be substituted as soon as possible.

4. **Endocarditis.**—That this disease is of bacterial infective origin seems to be sufficiently established. The infecting bacterium is thought to be in most cases *streptococcus fæcalis*, but *streptococcus pyogenes* and *staphylococcus pyogenes aureus* (Testi, 10, p. 67) have also been shown to be causes. So far, all efforts to arrest the disease by means of therapeutic immunization have proved unsuccessful.

5. **Diabetes Mellitus.**—The possibility of the application of therapeutic immunization has already been considered in Chapter X., section 14.

6. **Actinomycosis.**—This disease has now been successfully treated by therapeutic immunization. The matter is dealt with in Chapter X., section 14.

7. **Leprosy.**—Attempts have been made to treat this disease by vaccines. The subject is considered at some length by Allen (1, p. 160). He refers especially to the successful results obtained by Rost (*Indian Medical Gazette*, 1911-12) with a vaccine prepared from a fresh broth culture of the bacillus.

8. **Glanders.**—Allen (3) states that six cases of this disease have been recorded in which treatment by therapeutic immunization against *bacillus mallei* was tried, in three, with complete success. Trouble has been experienced on account of the toxicity of the vaccine and the occurrence of an intense local mallein reaction. An initial dose of 2,000,000 has been recommended.

CHAPTER XII

SOME CAUSES OF FAILURE OF THERAPEUTIC IMMUNIZATION

This subject has already been touched upon in the first chapter in considering the reasons for which therapeutic immunization is so little valued by the medical profession. If the reader has studied the intervening pages, his respect for this method of treatment has, I hope, been considerably enhanced, and he may be sincerely anxious, in employing it further, to avoid mistakes and to secure good results. It may therefore serve a useful purpose if, in this last chapter, I summarize what seem to me to be the most common causes of failure.

I think that, if those who condemn vaccine treatment on the ground of their own experience understood the real causes of their failures, they would, as persons of intelligence, admit that the blame lay, not with the method, but with themselves, in having based their procedure upon inaccurate and wholly insufficient data. It has too often been assumed that no special knowledge, beyond that acquired from the ordinary medical curriculum, is necessary in order to qualify a doctor to carry out therapeutic immunization. The fact is that little that is of practical value has, until recently at least, been taught on the subject in the medical schools, and the text-books of bacteriology, splendidly as they serve their own purposes, are inadequate for those of instruction in vaccine therapy, which requires the systematic application of methods still quite unorthodox, if accurate and successful work is to be done in any but the simplest type of case. Although much can be learned from several excellent special works already published, it is to be feared that few practitioners have taken the trouble to master the contents of even one of them, before embarking on the otherwise uncharted and perilous sea of the practice of therapeutic immunization. The necessity

for accurate bacteriological diagnosis is still imperfectly understood, and stock vaccines that it is hardly possible can correspond exactly to the infective conditions present in the case continue to be used by many in the belief that they are giving their patients every possible chance of benefit that vaccine treatment can offer. It is therefore not a matter for surprise, to those who have made a special study of the subject, that therapeutic immunization should appear, to a large proportion of those who have tried it, to be a method of treatment of little value, and, at best, very limited in its useful application. A long series of failures, an occasional brilliant success, and some very nasty accidents, sum up for many practitioners their experience of "vaccines."

1. **Unsuitability of Case.**—Too much is often asked of therapeutic immunization. It cannot make old people young; it cannot repair damage done to the tissues by years of bacterial attack; nor is it of any avail against the consequences of organic disease of vital organs. For example, I have been asked to apply therapeutic immunization (1) in the case of an old gentleman supposed to be suffering from chronic rheumatism, but whose malady was really advanced arteriosclerosis, (2) in a case of exophthalmic goitre in a patient who was found to have advanced chronic Bright's disease, and (3) in a case of pernicious anæmia in the last stages. Such cases are quite unsuitable for therapeutic immunization. Suppression of existing infections will not obviate the consequences of the grave organic lesions from which the patients are suffering.

2. **Defective Bacteriological Analysis.**—This is, I believe, the most common cause of unsuccessful therapeutic immunization. Bacteriological examinations carried out without blood media, and without the application of suitable anaerobic methods, can be complete only in the simplest cases. Lack of proper criteria for the differentiation of species is also often a cause of error that is fatal to success. For example, those who are content to slump all the chain-forming cocci in the one category of streptococci must frequently fail to immunize against one or more of the chief pathogenic species, especially in cases of infection of the respiratory and alimentary tracts. Infections of primary

importance as etiological factors may, and at present commonly do, pass undetected, such as those by *bacillus influenzæ*, pneumococci, and anaerobic strains of gonococci, of *streptococcus pyogenes*, *streptococcus fæcalis hæmolyticus*, diphtheroid bacilli, streptothrices and of *micrococcus catarrhalis*.

A fault of bacteriological diagnosis by no means rare is one that is liable to be made by the most skilled investigator. It is the failure to detect, at the first examination, some important pathogenic bacterium of delicate growth, the colonies of which have been obscured, or altogether suppressed, by the profusion of colonies of more vigorous bacteria. In cases of mixed infection in which steady improvement does not occur, a supplementary examination should always be made. After the more vigorous species have been in some measure suppressed, it is generally easy to detect any bacterium that has thus been missed.

3. **Faulty Vaccines.**—The error may be in the preparation (for example, subjection of the emulsion to excessive heat), in the standardization, or in the composition of a compound vaccine.

4. **Wrong Dosage.**—The dose may be insufficient to produce stimulation of the patient's defences. I have known this to occur from simple neglect to shake up the bottle or tube containing the emulsion, before measuring out the dose. Sometimes the dose is not increased enough with successive injections. In other instances, the doses are ridiculously small. For example, I was once asked to give a vaccine dose to a lady who was visiting Edinburgh. She handed me a bottle of autogenous vaccine prepared from intestinal cultures and labelled "Coli 20, strept. 10." I suppose these figures indicated the number of millions in one cubic centimetre. I estimated that a dose of 1 c.c., which had never been exceeded in the course of the patient's treatment, contained about 0·003 mg. of each of the two elements. It was not surprising that the patient had never experienced any disturbance from any of the doses, and that the intestinal trouble from which she had sought relief was no better.

The giving of excessive doses, especially in hypersensitive

cases, is probably a still more common cause of failure. Every injection makes the patient worse, and the blame is laid to the charge of the method of treatment. The significance of hypersensitiveness, and the proper method of dealing with it, have already been considered.

5. **Relapses from Natural Causes.**—In the middle of a course of immunization, especially against chronic infections of the respiratory tract, a patient may suddenly have an acute attack of the malady for which he is seeking relief. It will almost constantly be found that the cause of such a flare-up is a chill. It must be understood that, until the infective foci have been cleared of pathogenic bacteria, there will be liability to sudden fresh invasion from this cause, which, as already maintained, temporarily suspends the defensive action and permits rapid multiplication of the bacteria lying in the tissues. When the anti-bacterial forces come into action again, most of the new invaders are generally destroyed; much toxin is consequently liberated and the patient suffers from the usual effect of toxic action in and around the infective focus. When these facts are understood a relapse of this kind need be regarded as nothing more serious than a stumble in a winning race. Nevertheless, I have known such an occurrence so to discourage both the patient and the doctor that treatment was stopped, as being evidently unavailing.

6. **Acquirement of New Infections.** — I have observed numerous examples of this occurrence. Some persons who have long suffered from chronic infection, especially of the respiratory tract or of the fauces, are very liable to contract new infections, and it must be admitted they seem specially apt to do so just when the old infections have been more or less completely eradicated. In such instances, a relapse appears to have occurred just when it was thought that success had been achieved. If a relapse cannot be accounted for by a chill, it is always advisable to make a fresh bacteriological examination. For example, a patient who was being treated for chronic infections of the nasopharynx and bronchi by *streptococcus pyogenes*, *streptococcus anginosus* and an anaerobic *micrococcus catarrhalis*, and who was improving steadily, had a sudden recurrence of all his symptoms. I

found that he had acquired a new infection by an aerobic *micrococcus catarrhalis* that was at the time causing an epidemic of common colds.

7. **Infection of Closed Cavities.**—Therapeutic immunization is of little avail against infection occurring in the walls of cavities from which the inflammatory exudation is unable to escape. For example, a lady who suffered from early rheumatoid arthritis was treated for a pneumococcus infection of the gums without success. It was discovered that one antrum was filled with fluid. This was allowed to drain away by opening up an old sinus in the floor connected with an alveolus. Pneumococci were abundant in the discharge. The patient afterwards made a good recovery.

8. **Saturation by Toxins.**—This occurs especially in some cases of severe diphtheroid bacillus infection. The patients seem to have no power of response to corresponding autogenous vaccines.

9. **Persistent Hypersensitiveness.**—This is always an indication of the presence of very extensive infective foci. It is typically seen in some cases in which there are adenoid growths in the nasal passages.

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Printed in Great Britain by the Riverside Press Limited
Edinburgh